



Mining Minnows and Building Models

**An Integrated Systems Biology Approach to Link Mechanism
of Action to Ecologically-Relevant Outcomes**



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Mining Minnows

A short introduction



1. Linking across biological levels of organization



2. Understanding compensatory responses

3. Making the most of genomic data





Strategic Objectives:

1. Improve understanding of the linkages in the continuum between the source of a chemical in the environment and adverse outcomes
2. Provide predictive models for screening and testing
3. Improve quantitative risk assessment

- Computational biology:

- e.g. genomics, proteomics, metabolomics, bioinformatics

- Systems biology:

- Application of reasoning and mathematical modeling to understand biological phenomena.

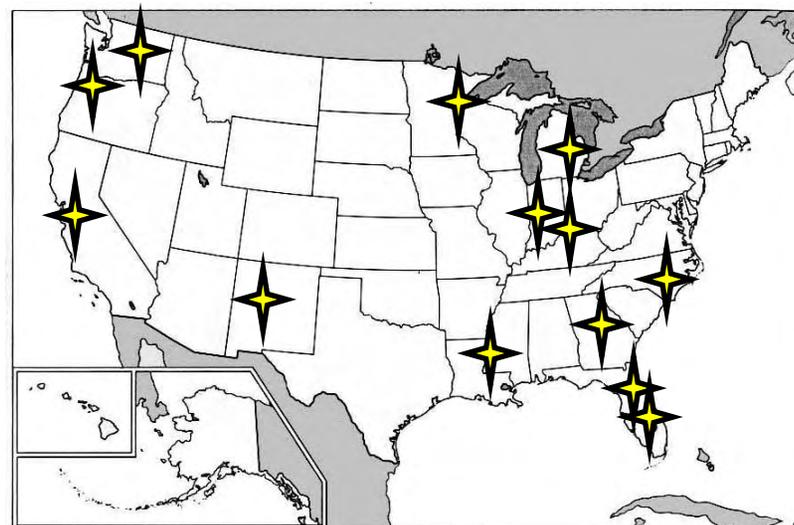
- Computational chemistry:

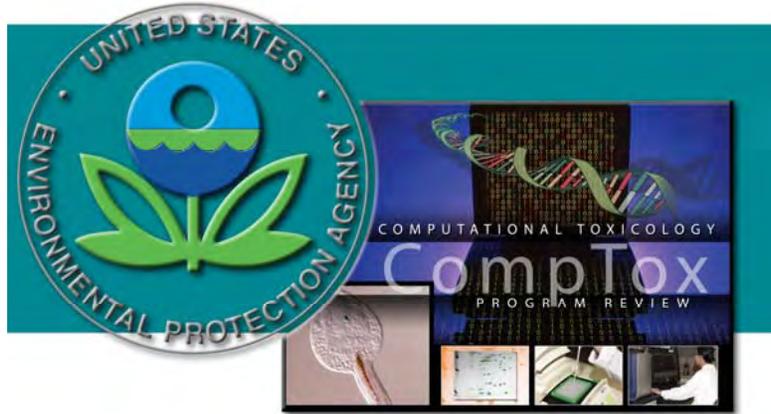
- Physical/chemical modeling at the molecular level

For more information: <http://www.epa.gov/comptox/index.html>

Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models

- USEPA – Cincinnati, OH
 - D. Bencic, M. Kostich, I. Knoebl, D. Lattier, J. Lazorchak, G. Toth, R. Wang,
- USEPA – Duluth, MN, and Grosse Isle, MI
 - G. Ankley, E Durhan, M Kahl, K Jensen, E Makynen, D. Martinovic, D. Miller, D. Villeneuve,
- USEPA – Athens, GA
 - T. Collette, D. Ekman, J. Kenneke, T. Whitehead, Q. Teng
- USEPA-RTP, NC
 - M. Breen, R. Conolly
- USEPA STAR Program
 - N. Denslow (Univ. of Florida), E. Orlando, (Florida Atlantic University), K. Watanabe (Oregon Health Sciences Univ.), M. Sepulveda (Purdue Univ.)
- USACE – Vicksburg, MS
 - E. Perkins
- Other partners
 - Joint Genome Institute, DOE (Walnut Creek, CA)
 - Sandia, DOE (Albuquerque, NM)
 - Pacific Northwest National Laboratory (Richland, WA)
 - C. Tyler (Univ. Exeter, UK)



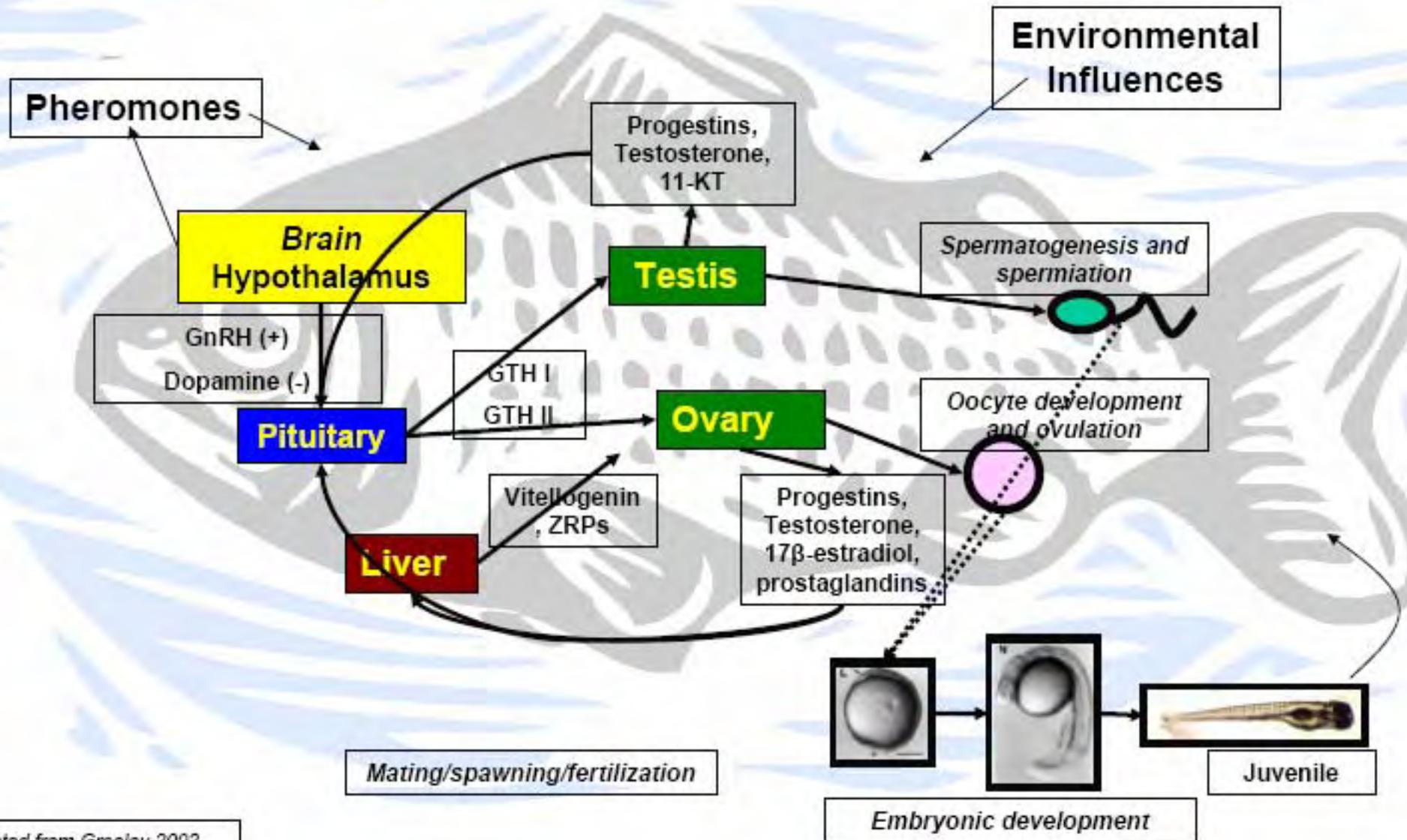


Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models

Research Goals

- Identify new molecular markers of effects of exposure to chemicals representing different MOA within the HPG axis of small fish models.
- Link molecular biomarkers to responses relevant for ecological risk assessment.
- Support development of integrated modeling approaches to use MOA as basis for predicting adverse outcomes.

Overview of Fish Reproduction



Compartment

Chemical "Probes"

Brain

Pituitary

Blood

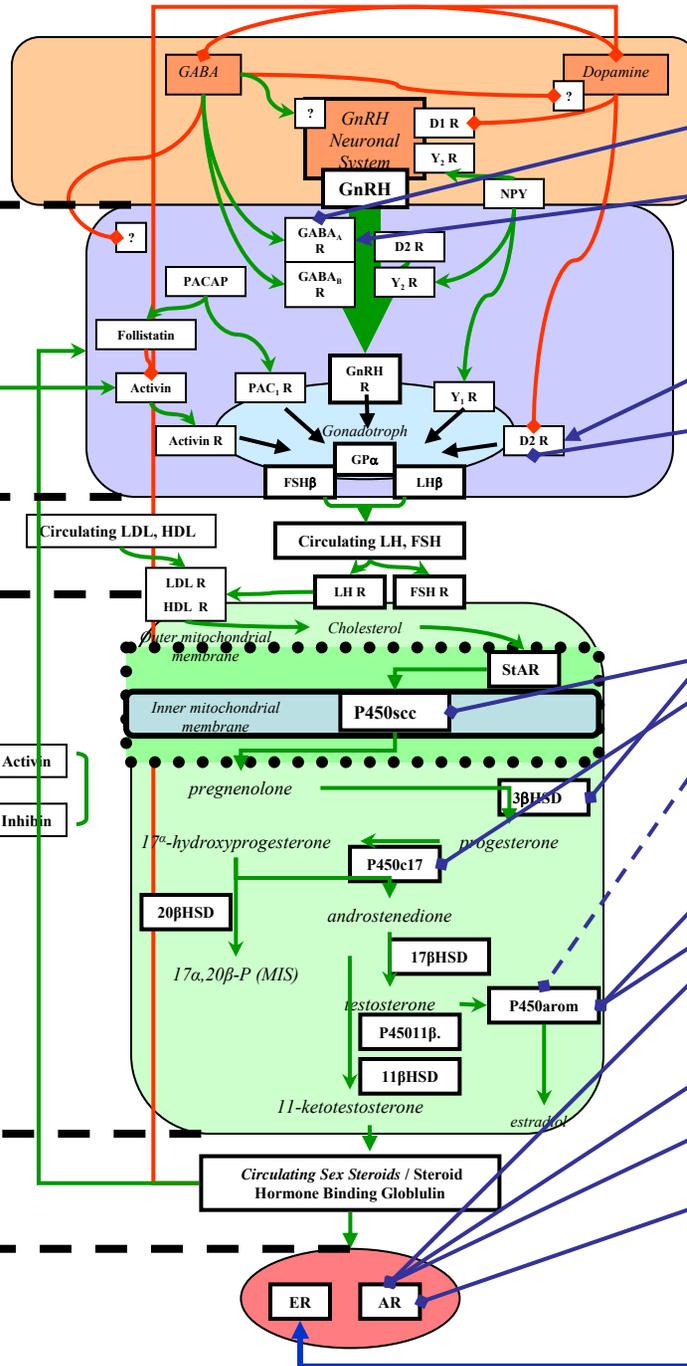
Gonad

(Generalized, gonadal, steroidogenic cell)

Blood

Androgen / Estrogen Responsive Tissues

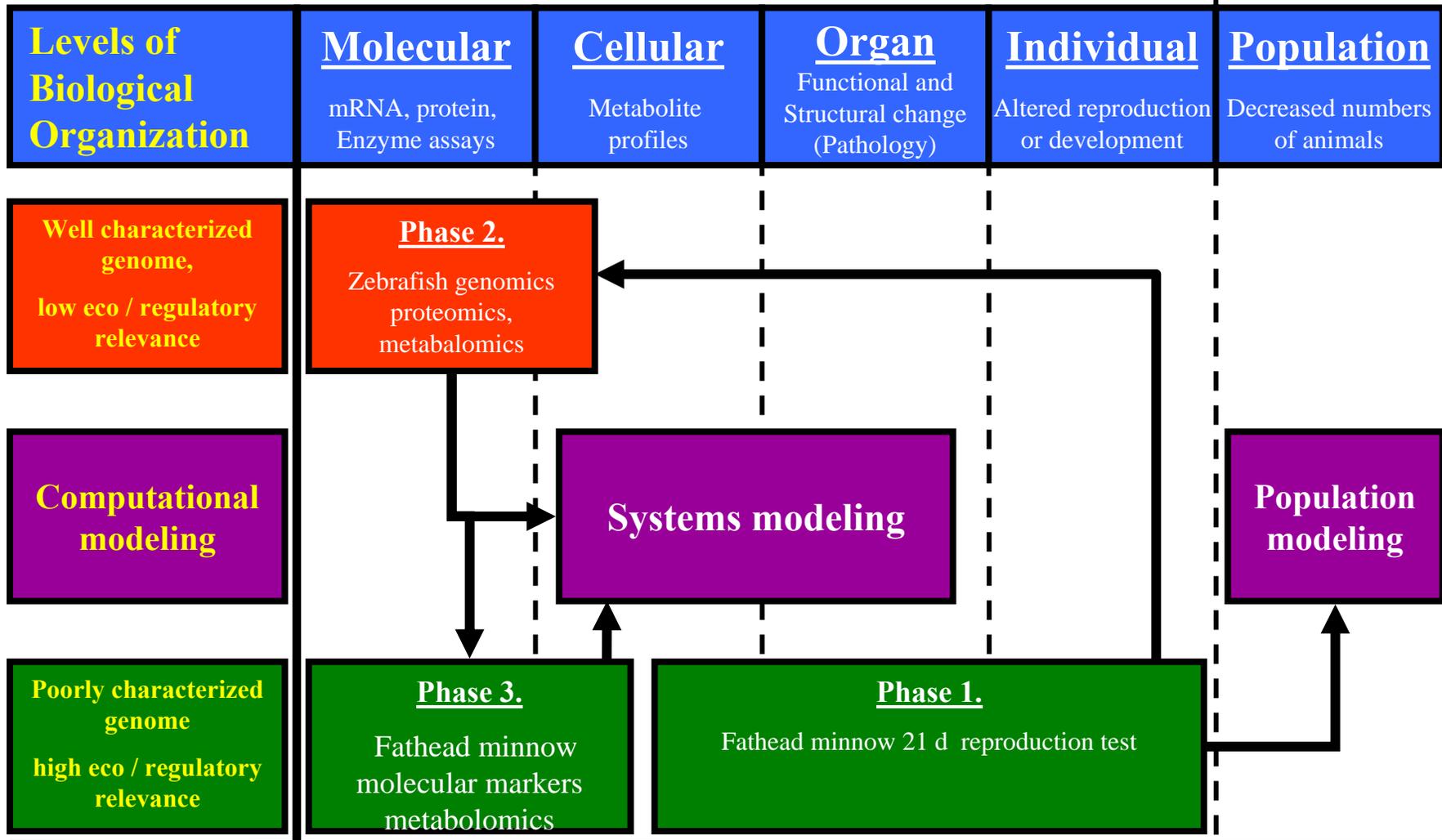
(e.g. liver, fatpad, gonads)



- 1 Fipronil* (-)
- 2 Muscimol* (+)
- 3 Apomorphine (+)
- 4 Haloperidol (-)
- 5 Trilostane (-)
- 6 Ketoconazole (-)
- 7 Fadrozole (-)
- 8 Prochloraz (-,-)
- 9 Vinclozolin (-)
- 10 Flutamide (-)
- 11 β -trebolone (+)
- 12 Ethinyl estradiol (+)

Figure 1. Conceptual Overview of Research

Increasing Diagnostic (Screening) Utility ← → Increasing Ecological Relevance



→'s Depict the flow of information

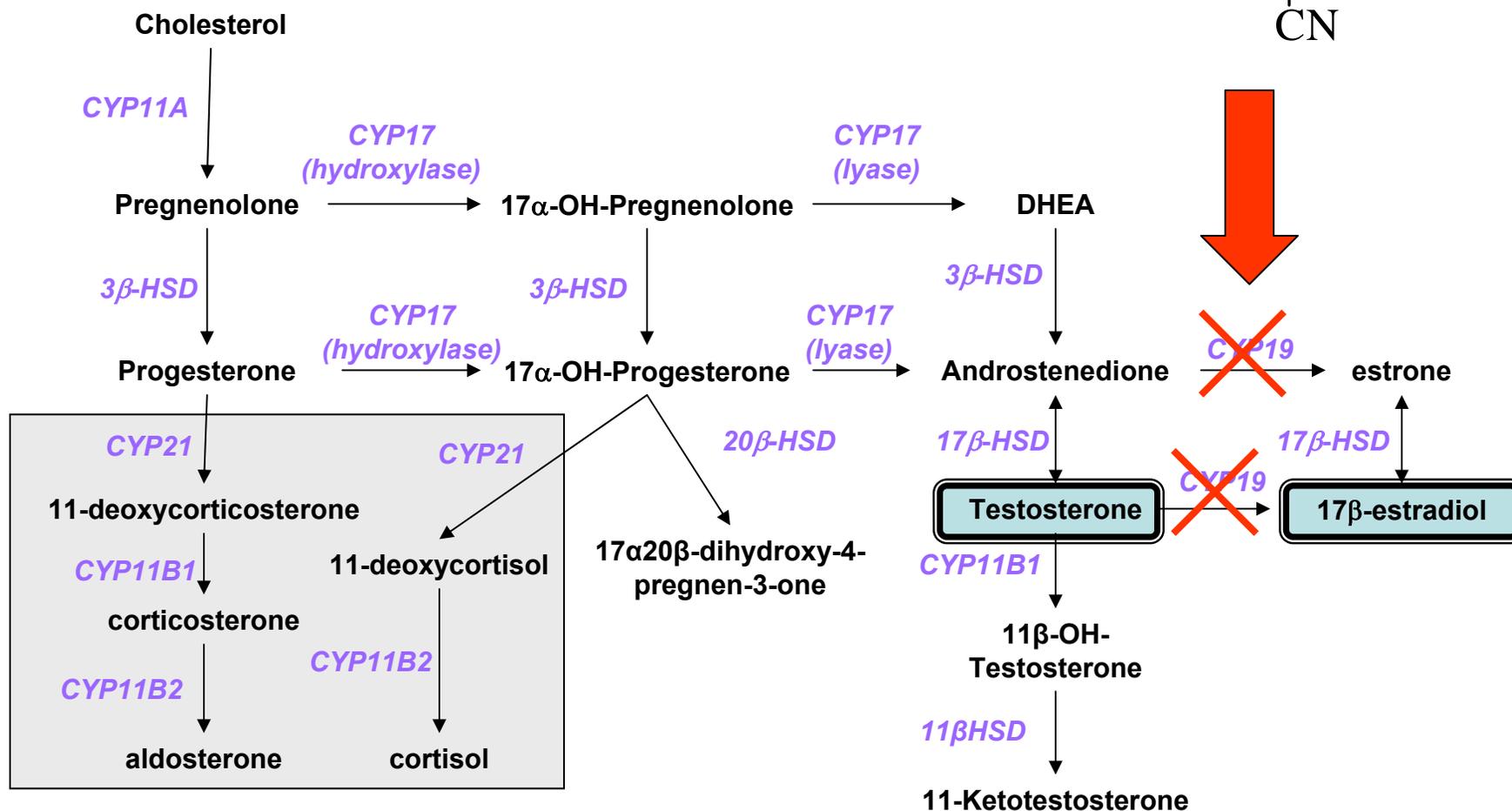
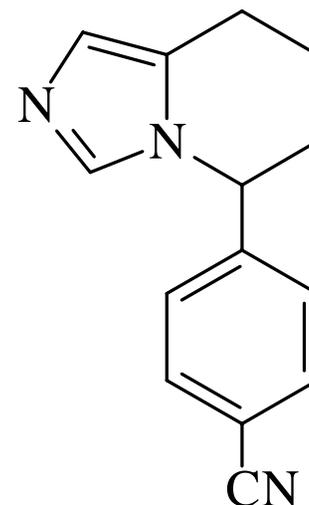
Mining Minnows



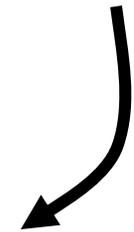
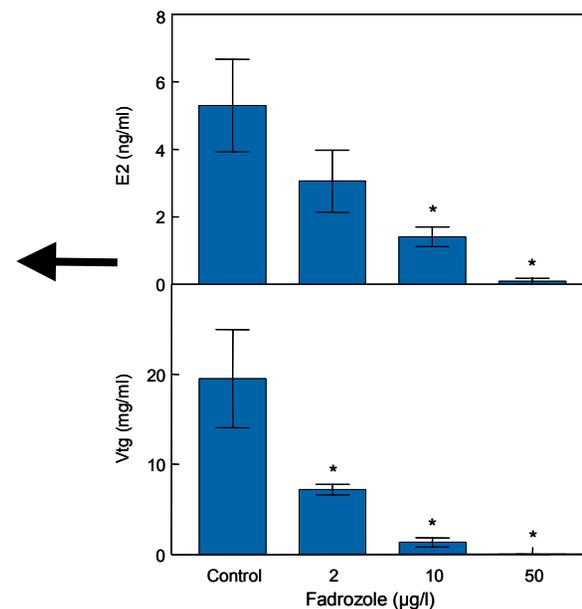
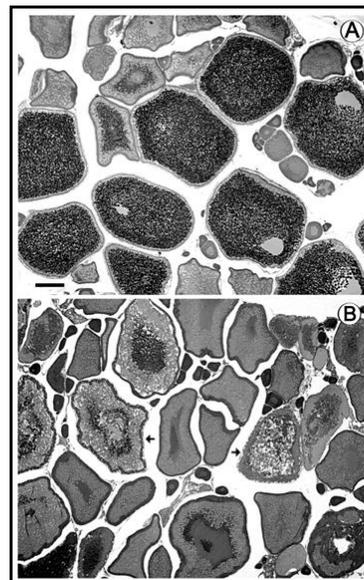
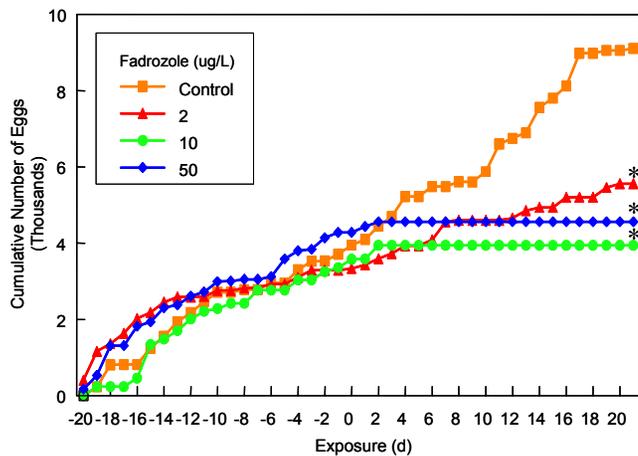
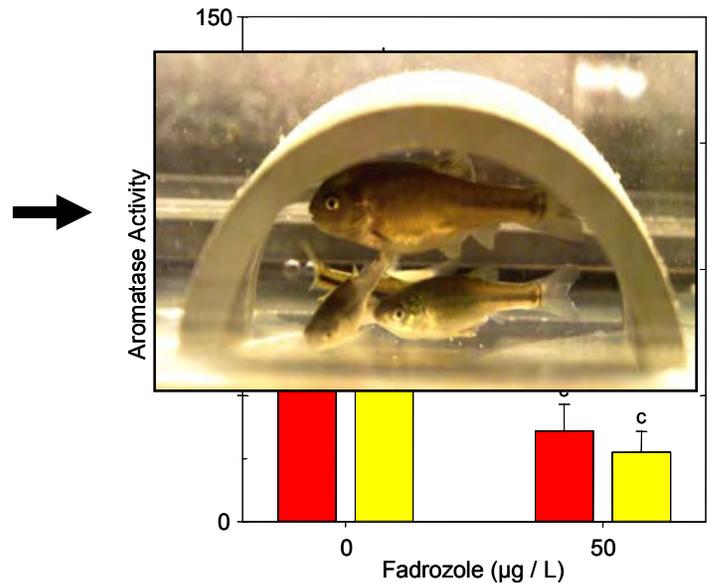
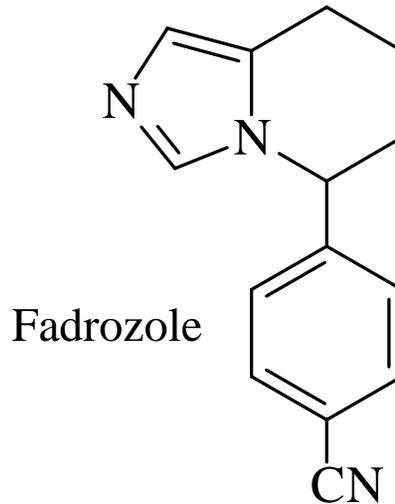
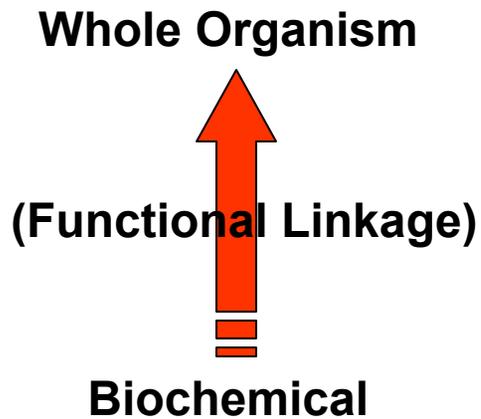
1. Linking across biological levels of organization

Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models

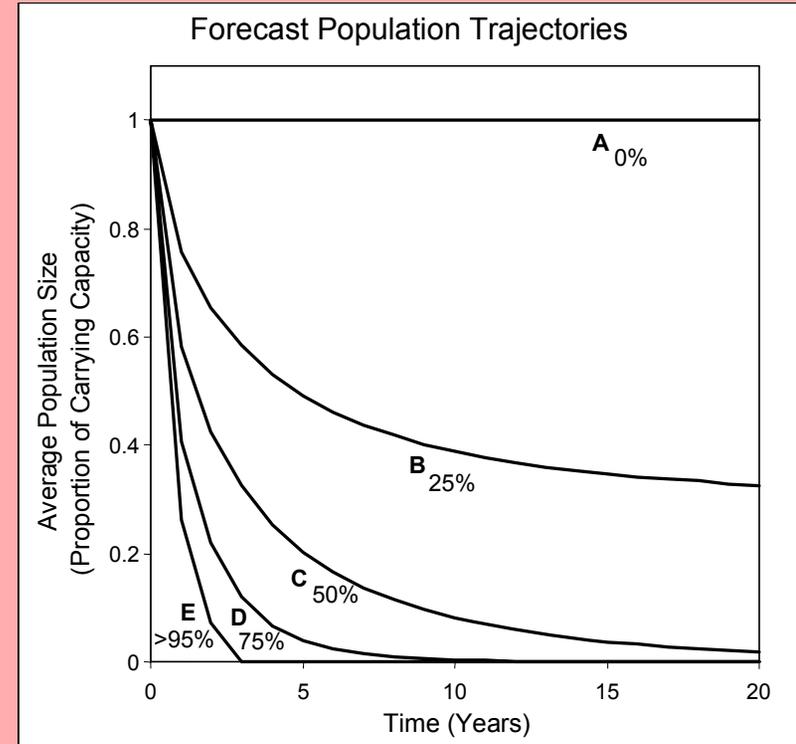
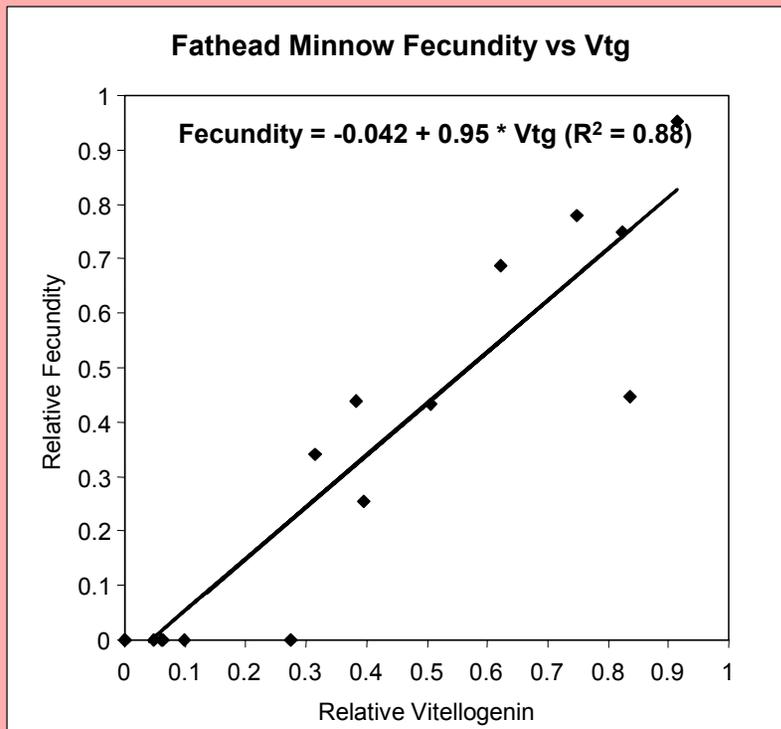
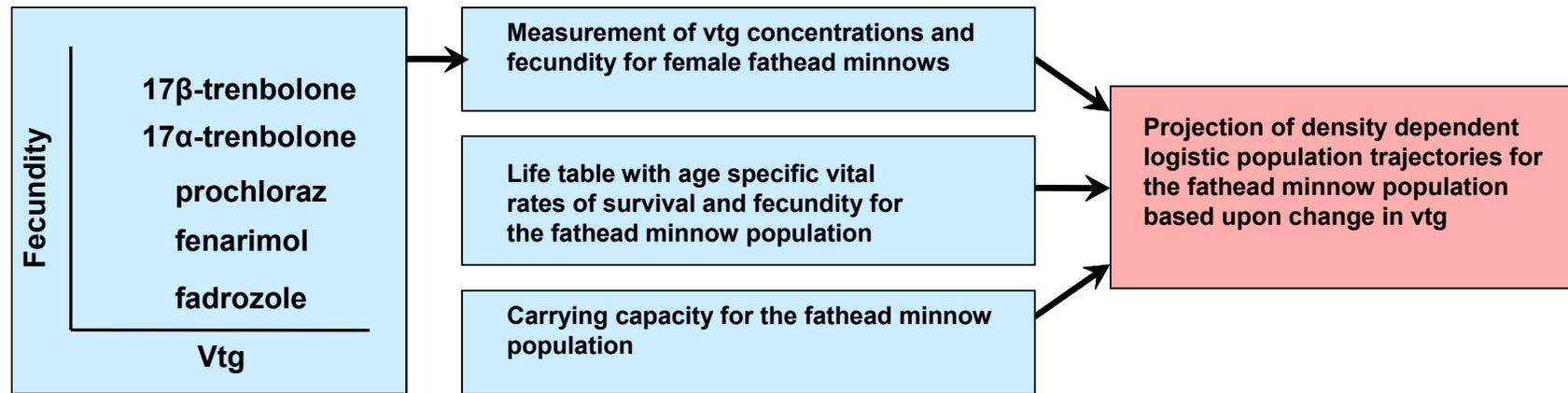
A Case Study with Fadrozole



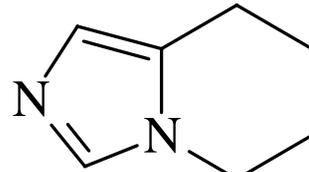
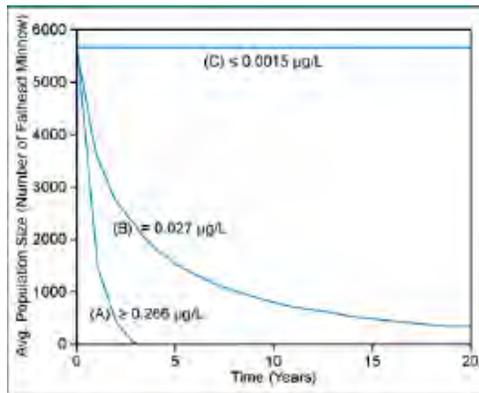
Effects of Aromatase Inhibition on Reproduction in the Fathead Minnow (21 d)



Forecasting Population Effects from Vtg Reduction



Effects of Aromatase Inhibition on Reproduction in the Fathead Minnow

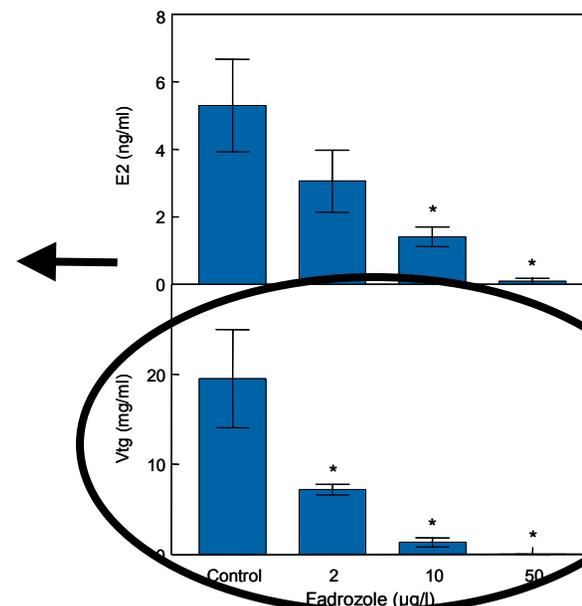
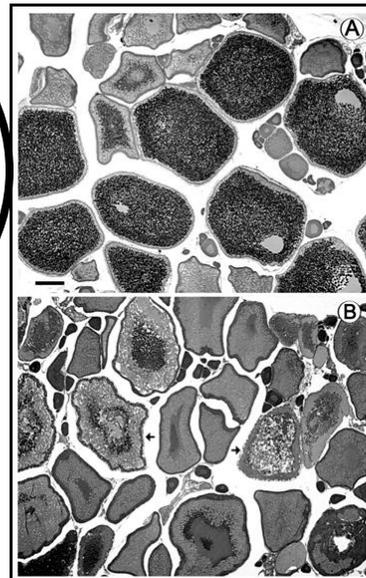
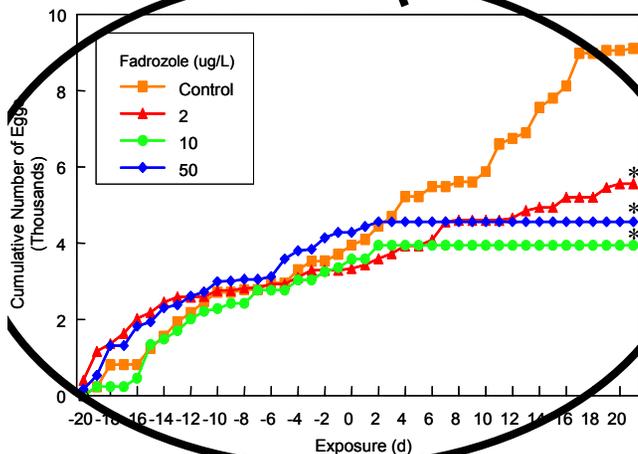
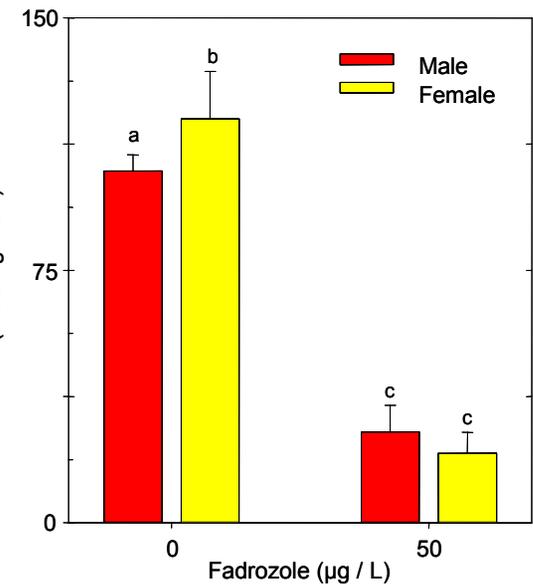


Population

Biochemical

Fadrozole (Functional Linkage)

Aromatase Activity ($\text{fmol/mg}^{-1} \text{hr}^{-1}$)



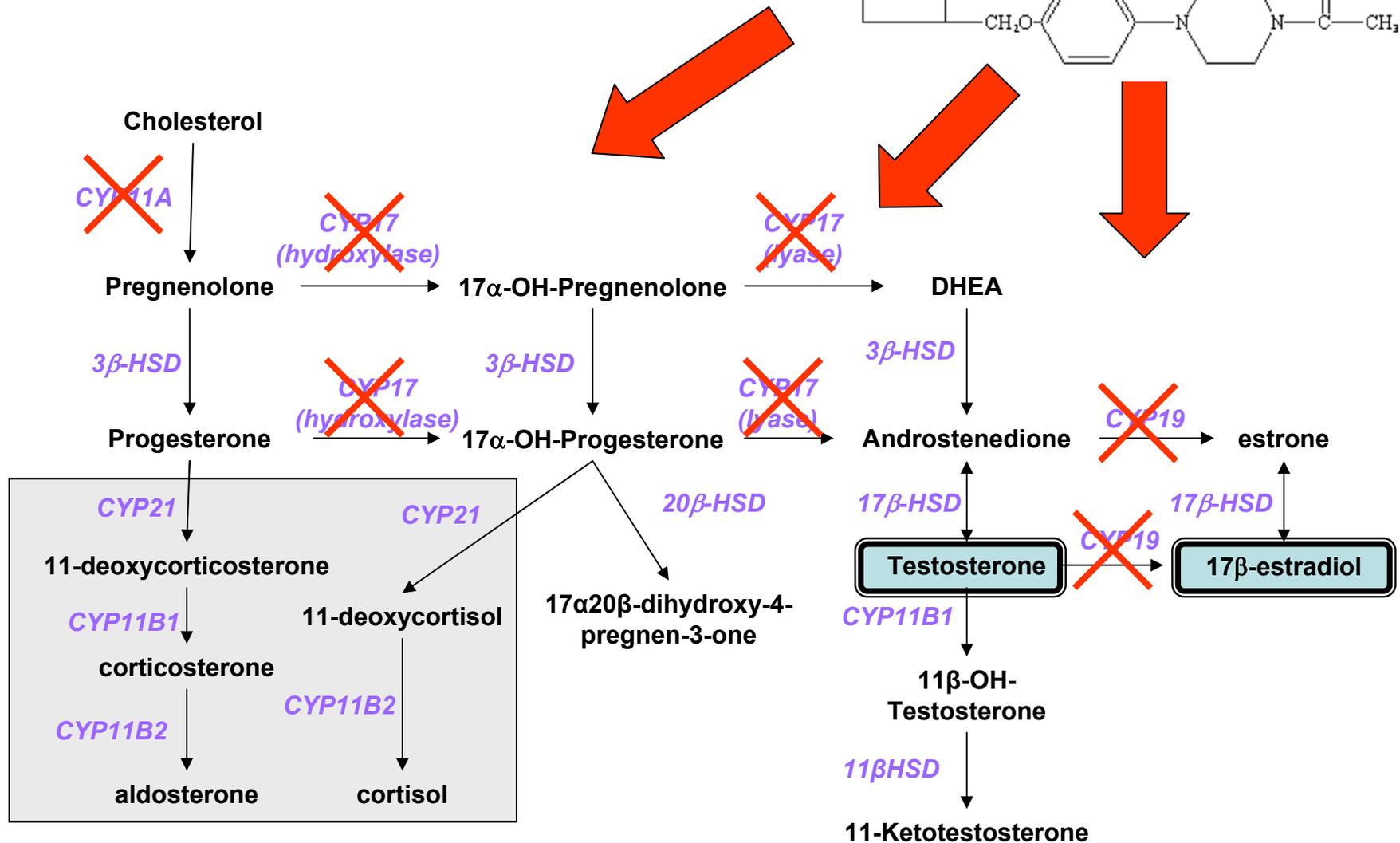
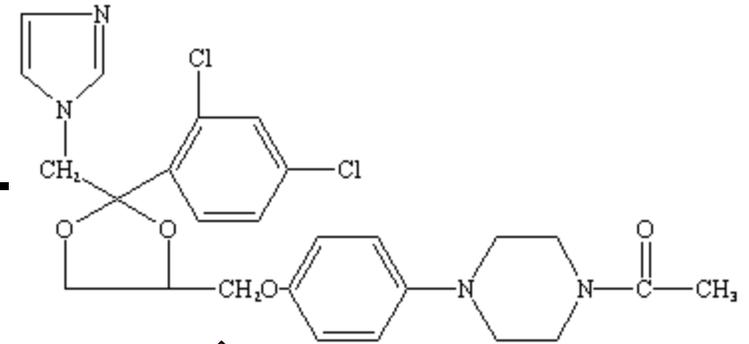
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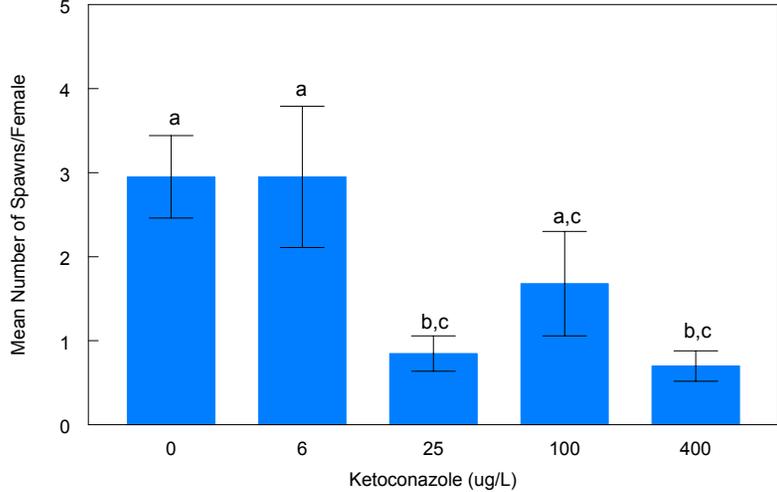
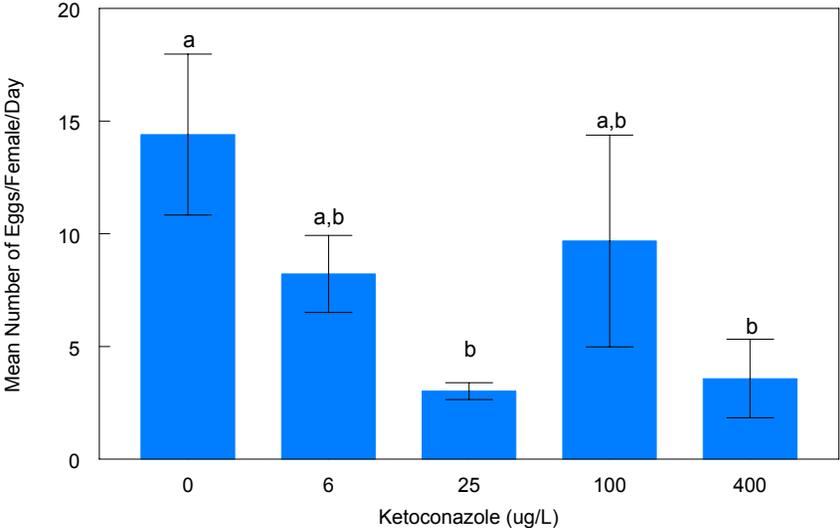
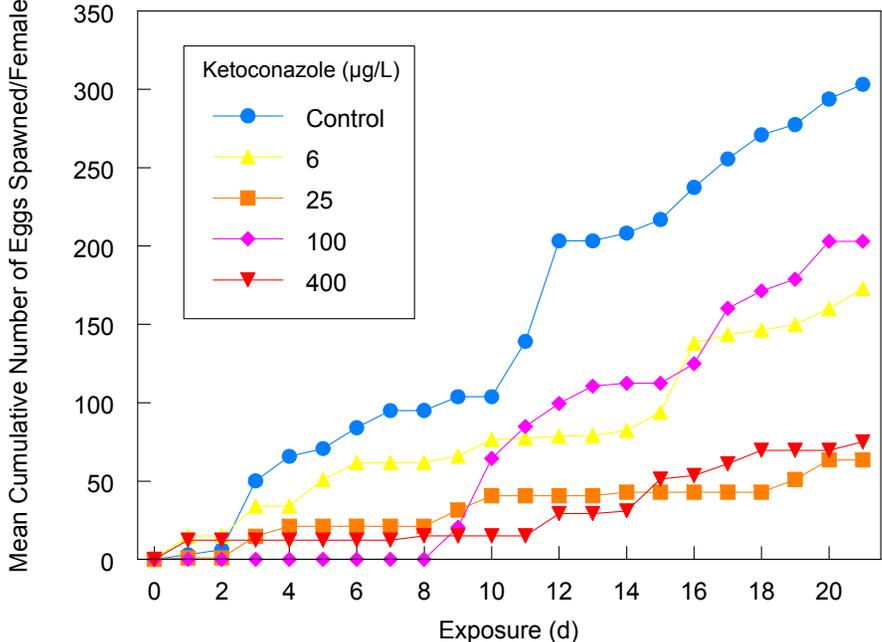
2. Understanding compensatory responses to chemical stressors.

Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models

Compensatory Responses to Ketoconazole



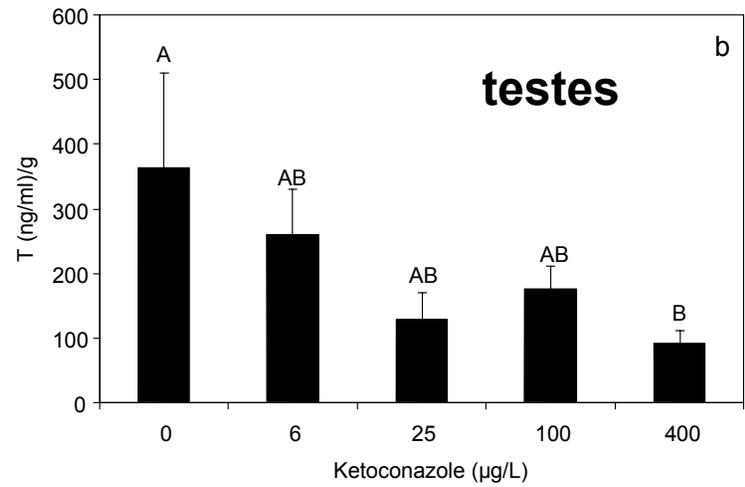
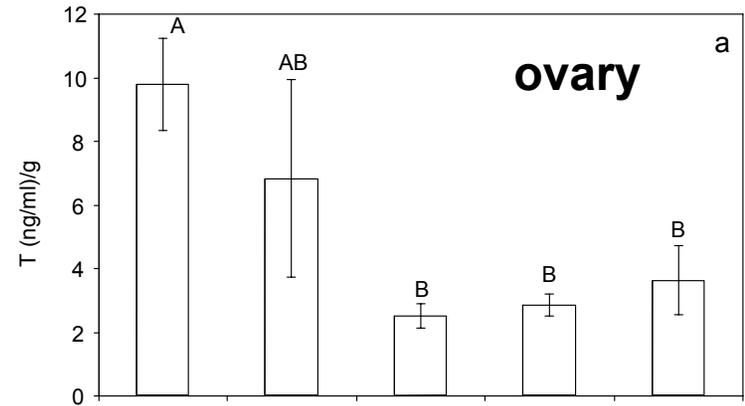
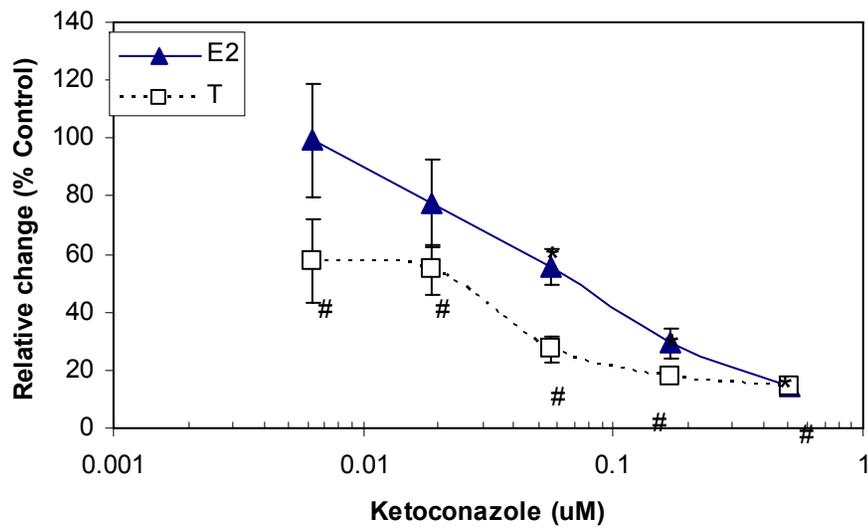
Ketoconazole: Effects on Fecundity



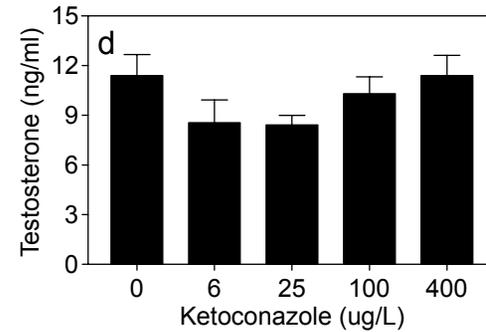
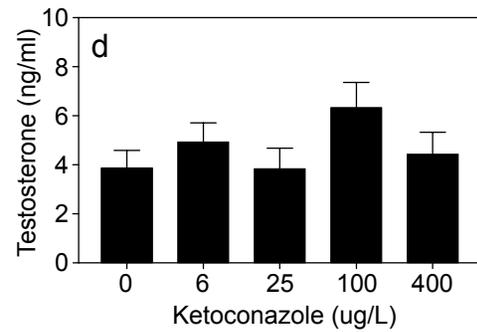
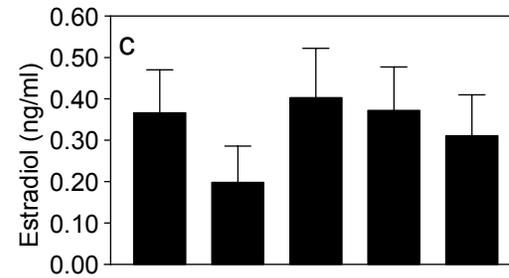
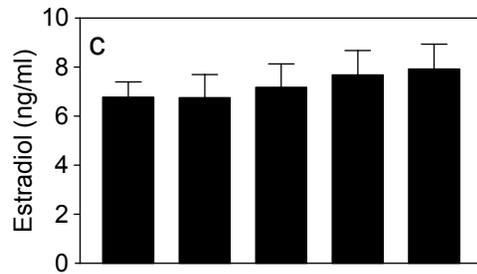
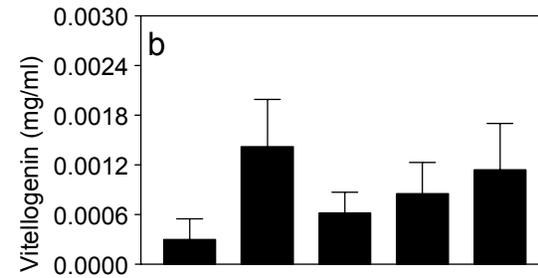
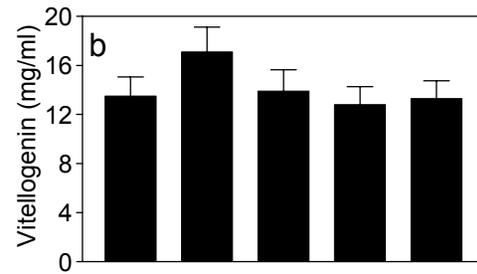
Effect of KTC on Steroid (E2 and T) production

In vitro

Ex vivo

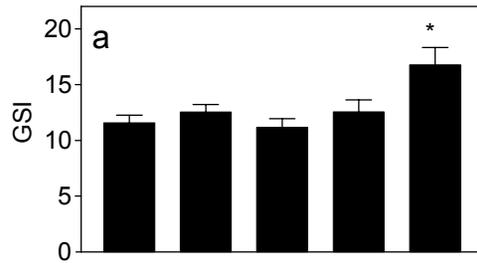


Ketoconazole: Effects on Plasma Steroids and Vitellogenin

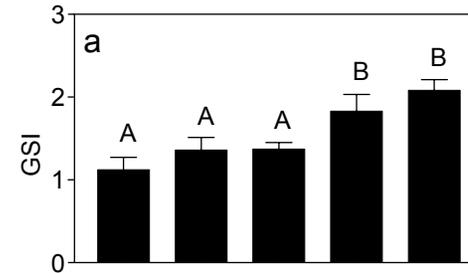


Female

Male

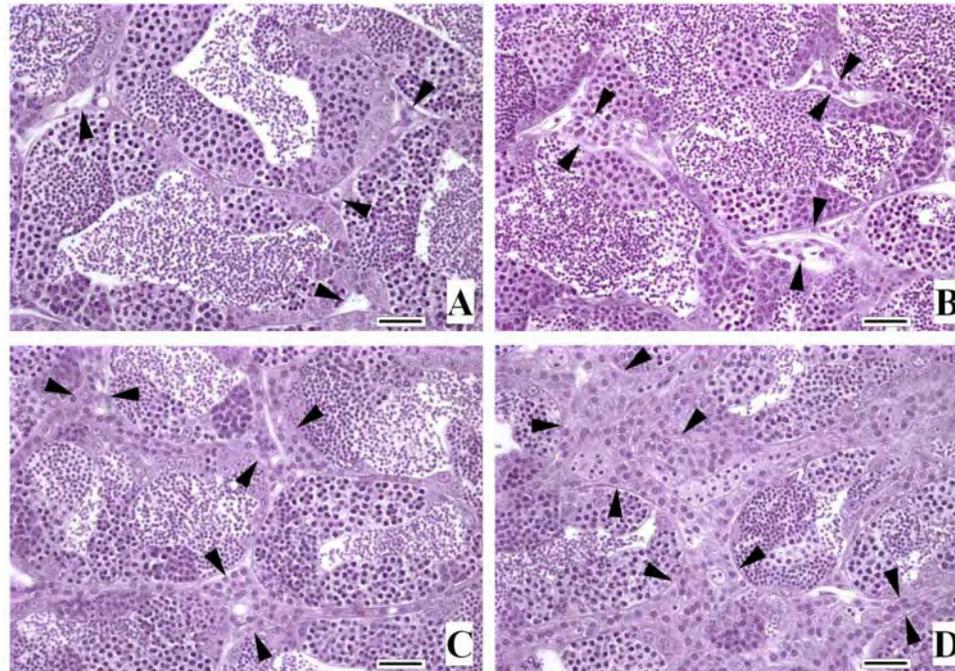


Female



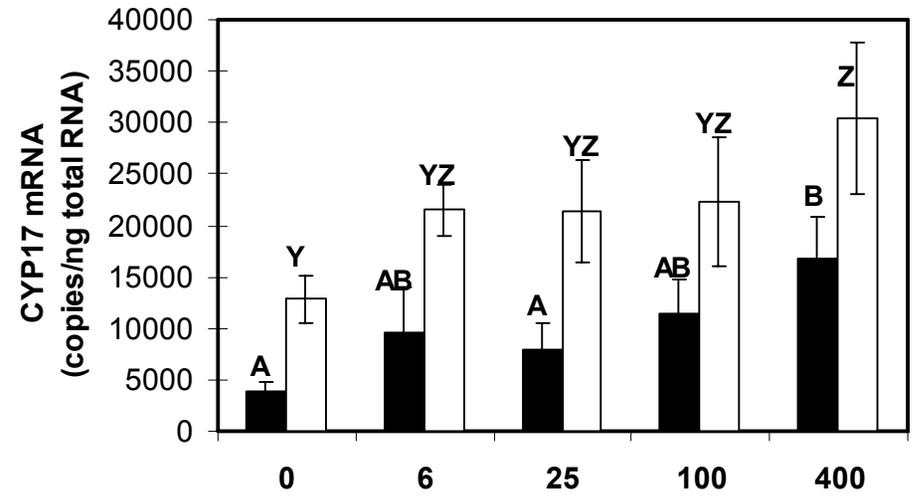
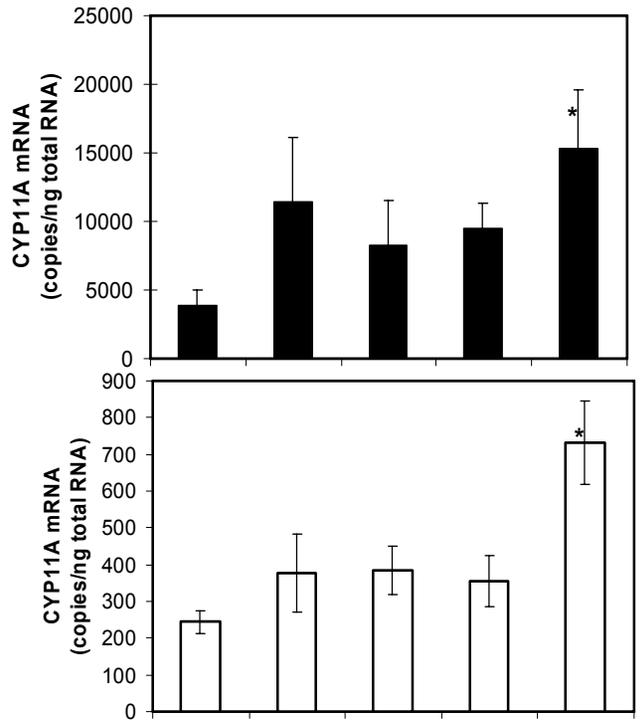
Male

Histopathology, male gonad

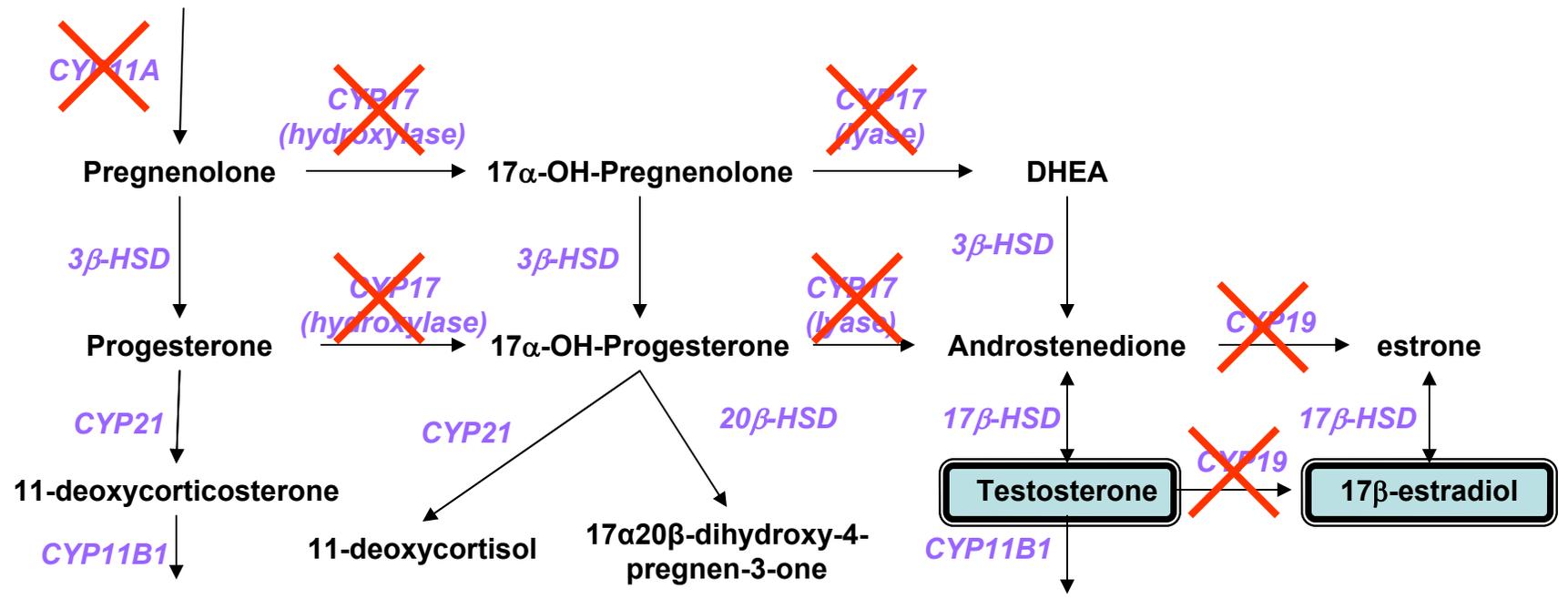


Proliferation of Interstitial Cells Involved in Steroid Synthesis

A, B = Controls; C= 6 µg/L; D= 400 µg/L



Cholesterol



2. Understanding compensatory responses to chemical stressors.

Time course experiments have shown evidence for compensatory responses at the metabolomic level as well (not shown)

For risk assessment: important to distinguish responses indicative of compensation from those leading to adverse effect.

Mining Minnows



3. Making the most of genomic data a three pronged approach

- a) Fingerprinting
- b) Hypothesis-driven
- c) Discovery-driven

Biological Responses and Genomics: An Overview

WHAT RESPONSES
ARE POSSIBLE

"omics" = totality

GENOMICS



INITIATION OF
RESPONSE



TRANSCRIPTOMICS

PHENOTYPE

WHAT DRIVES
THE RESPONSE

PROTEOMICS

THE METABOLIC
RESPONSE

METABOLOMICS

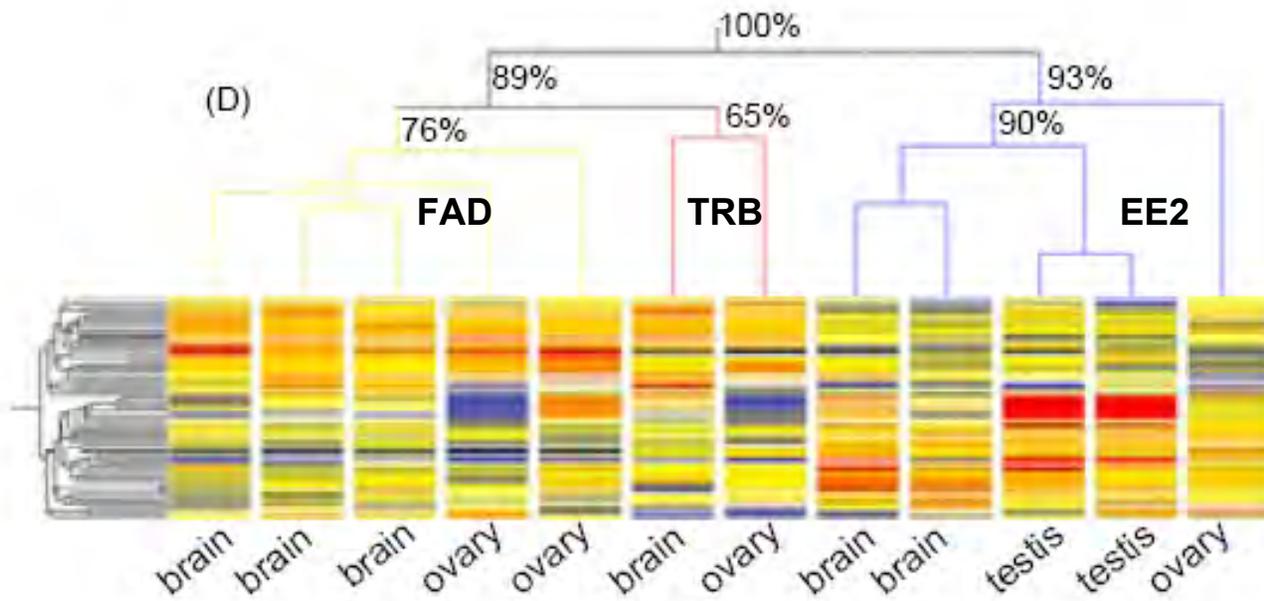
Adapted from Viant (2005)

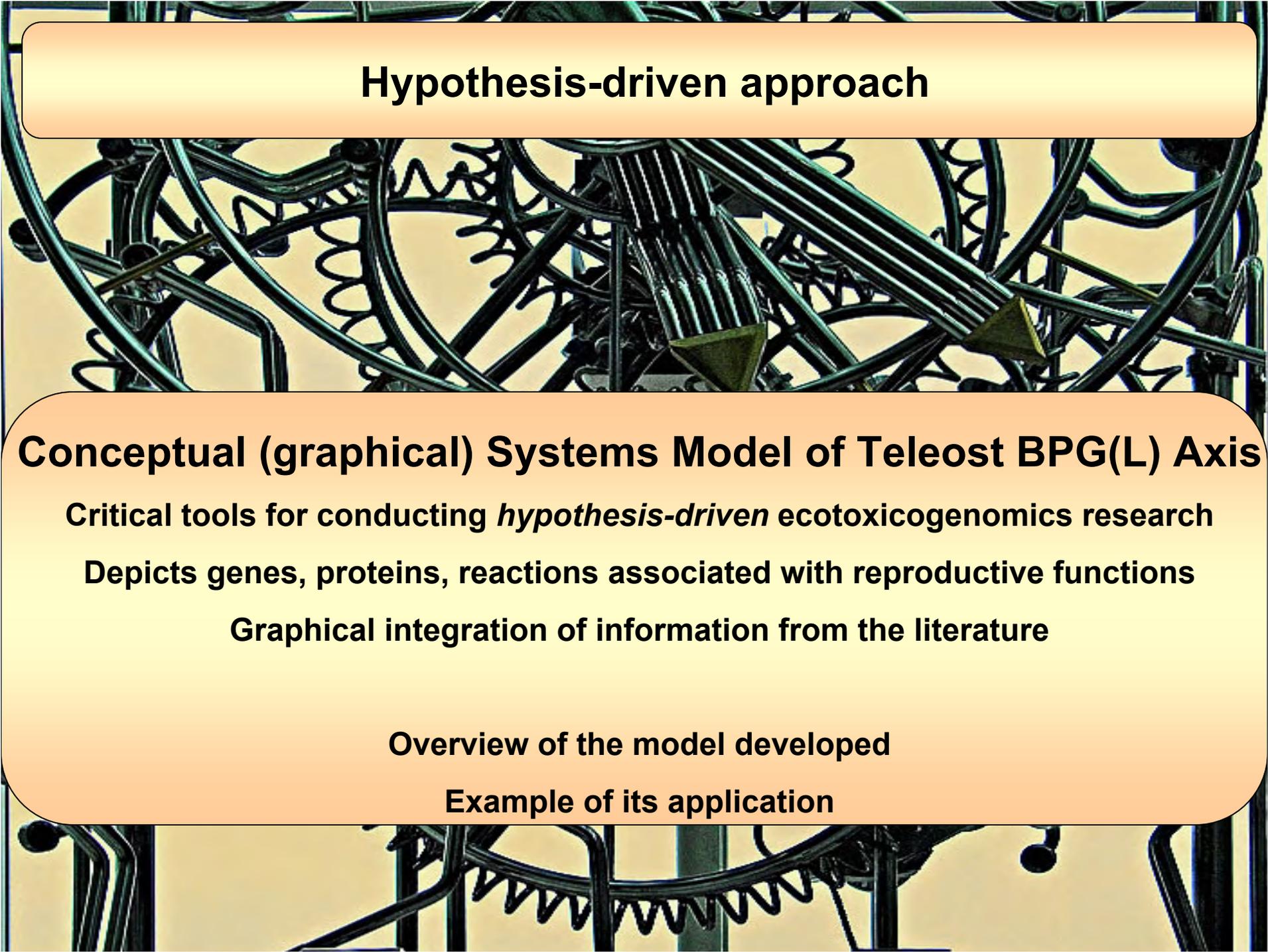


Fingerprinting approach

- Identify patterns and groups of responses that can serve as distinct fingerprints or classifiers for a given condition, chemical exposure, or mode of action.
- Mechanistic/functional understanding not necessary
- Extensive annotation is not necessary
- Not well equipped to determine what biomolecules do, how they interact, and their functional significance.
- May be useful for exposure assessment(s) and/or classifying chemicals by MOA

Fingerprinting approach





Hypothesis-driven approach

Conceptual (graphical) Systems Model of Teleost BPG(L) Axis

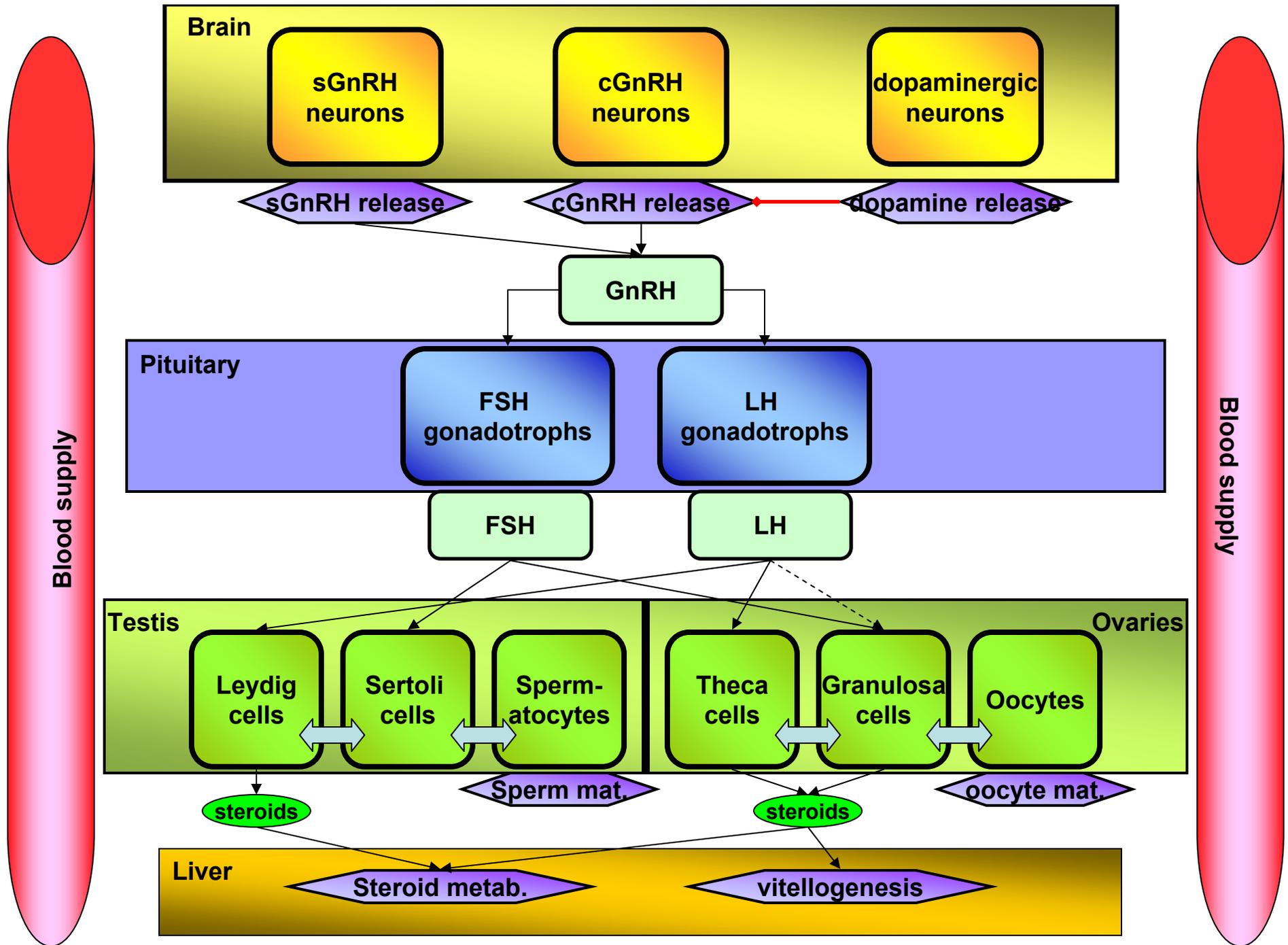
Critical tools for conducting *hypothesis-driven* ecotoxicogenomics research

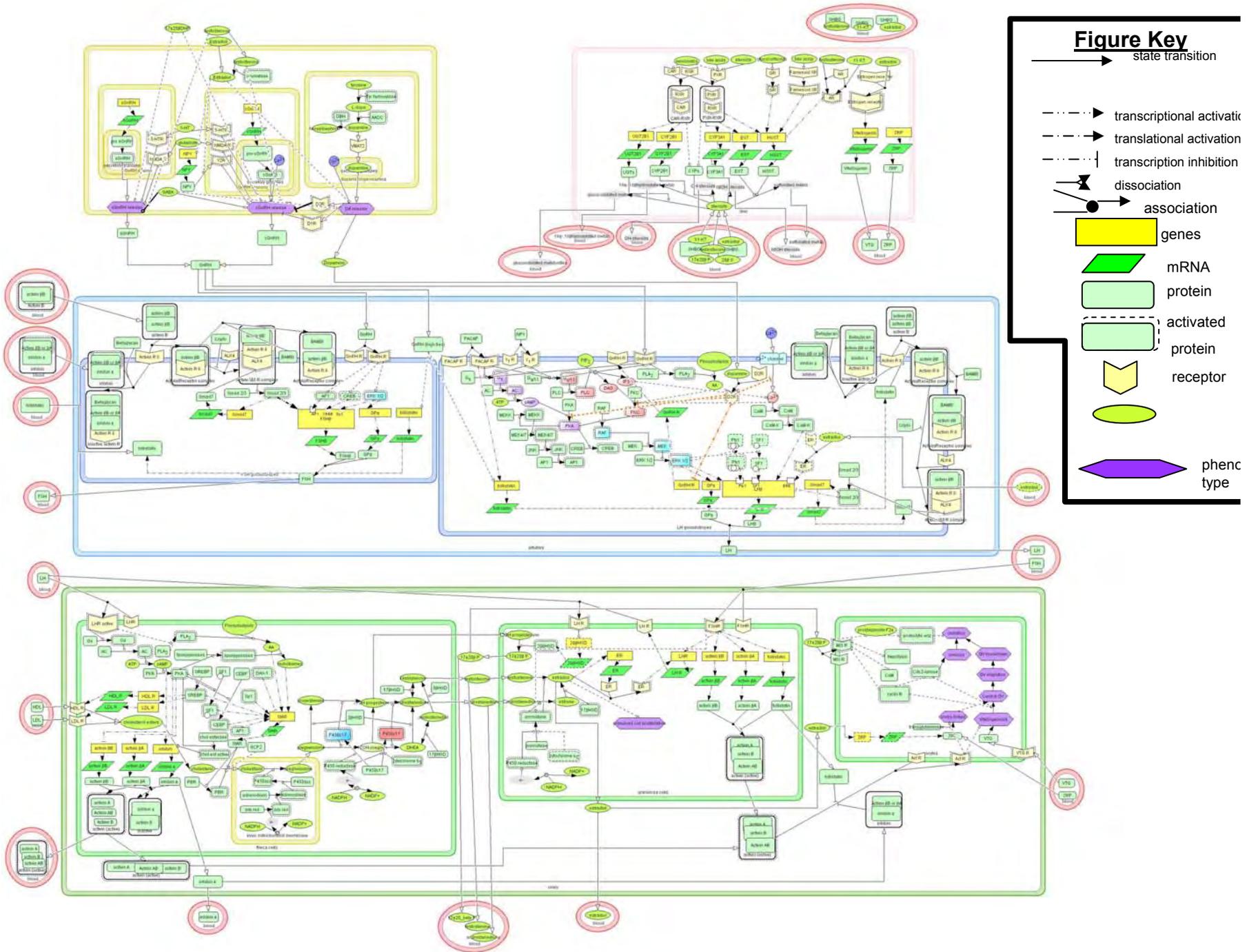
Depicts genes, proteins, reactions associated with reproductive functions

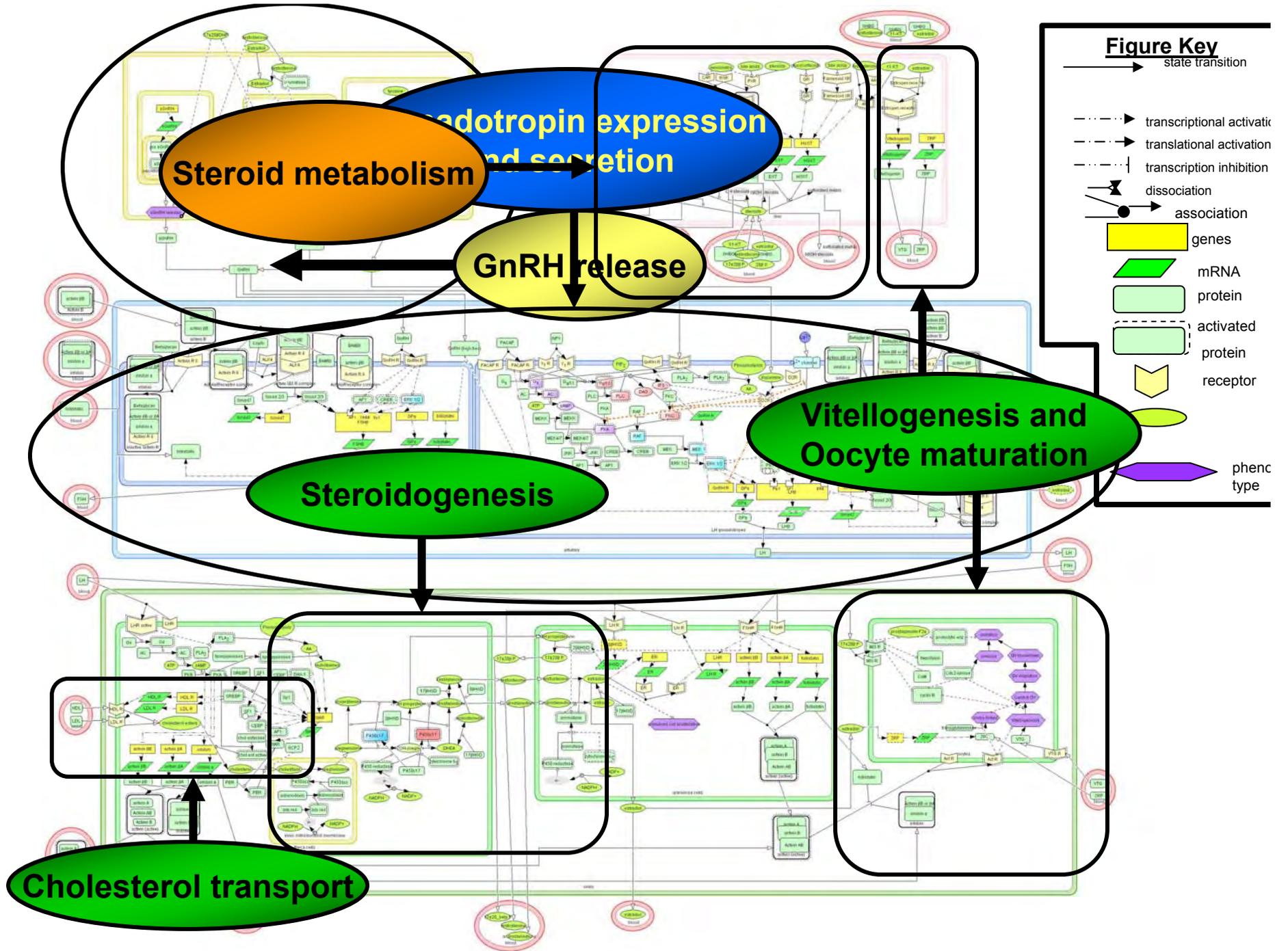
Graphical integration of information from the literature

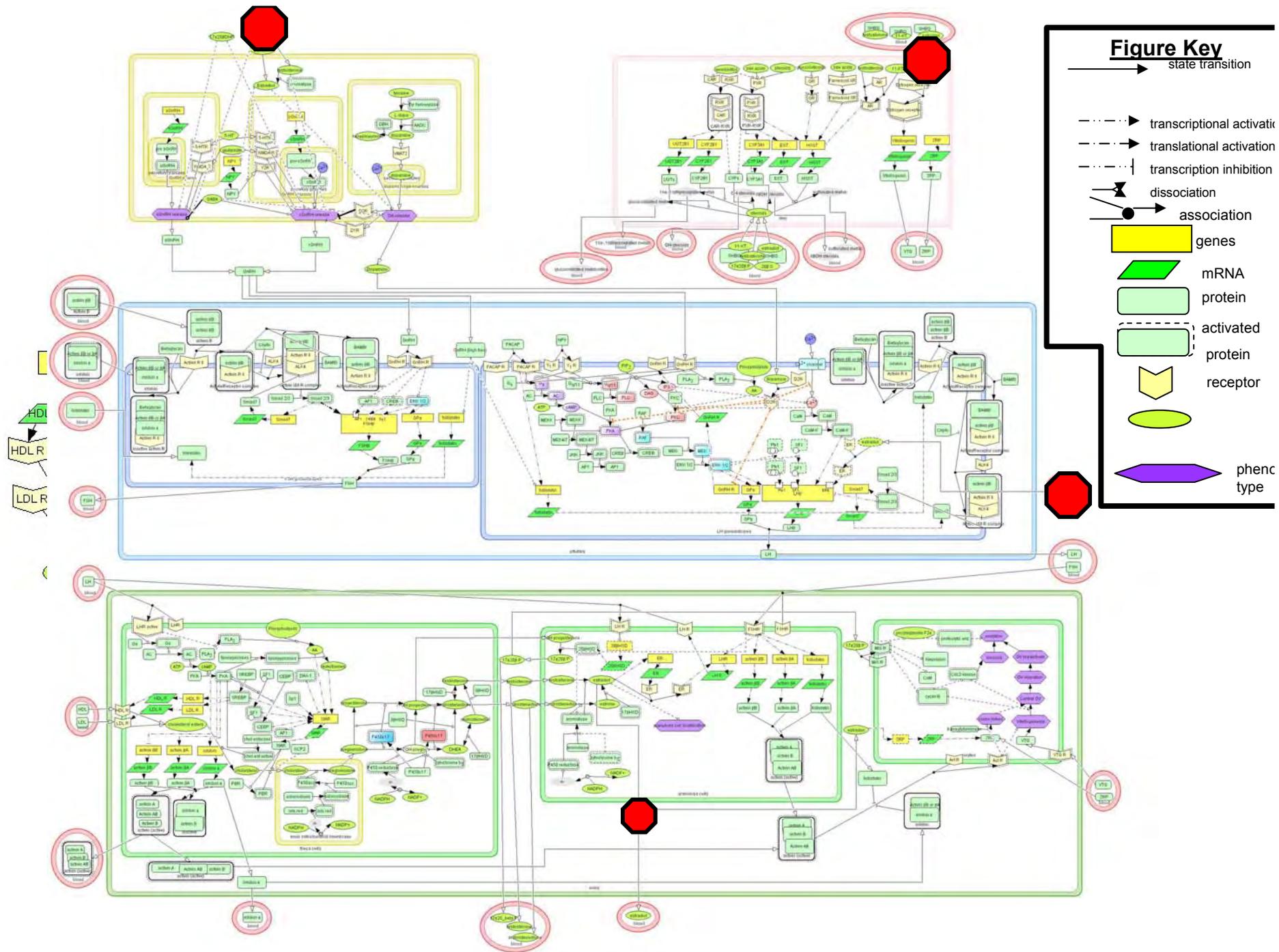
Overview of the model developed

Example of its application





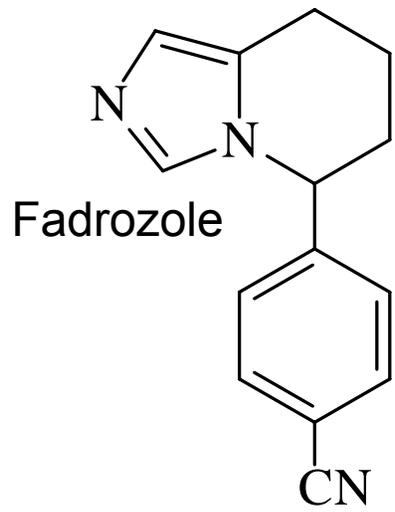




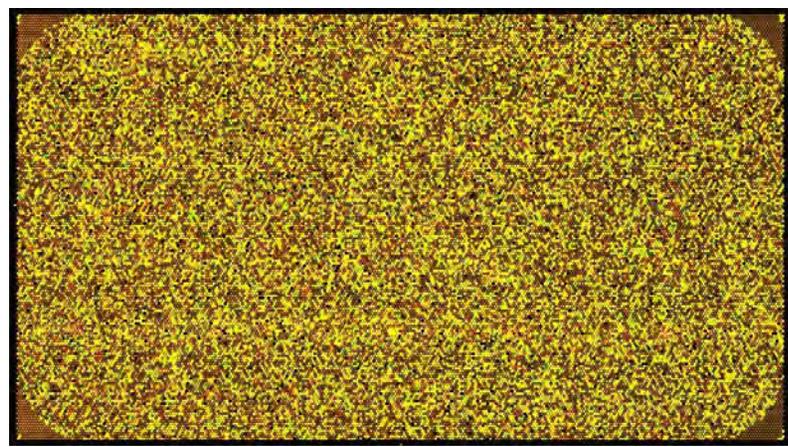
Steroidogenesis-related

Tissue	Gene ^{a*}	Fem Direct	Fem compensation
Ovary	CYP19A	▲	▲
	11βHSD	NC	NC
	17βHSD	NC	NC
	3βHSD	NC	NC
	CYP17	NC	▲
	20βHSD (carbonyl reductase)	NC	▼
	CYP11A (P450 _{scc})	NC	▲
	StAR	NC	▲
	LDL R	NC	▲
	Adx	NC	▲
	Adx reductase	NC	▲
	Ferredoxin/ Ferredoxin reduct.	NC	▲

Selected Pathway: Steroidogenesis-related Table
 Colored by: ZF fadrozole 48 and 96 h ovary, Default Interpretation
 Gene List: Steroidogenesis-related Table (16)



**22 K, oligonucleotide
microarray**

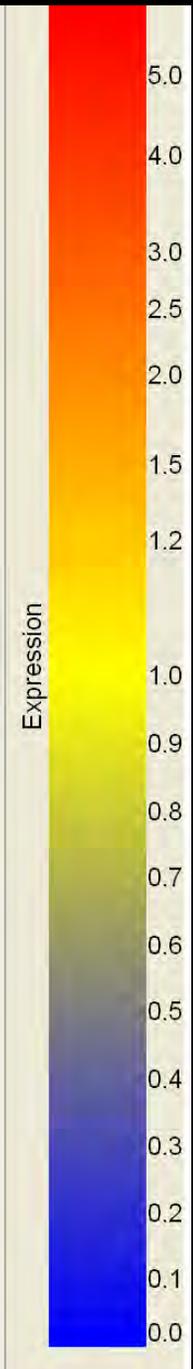


Steroidogenesis-related

Tissue	Gene ^{a*}	Fem Direct	Fem compensation	ZF probe id ^b or accession
Ovary	CYP19A	▲	▲	120366
	11βHSD	NC	NC	106783
				110126
				100202
	17βHSD	NC	NC	114681
	3βHSD	NC	NC	112736
	CYP17	NC	▲	108178
	20βHSD (carbonyl reductase)	NC	▼	120758
				104576
				116772
	CYP11A (P450 _{scc})	NC	▲	10102
				119670
	StAR	NC	▲	117295
	LDL R	NC	▲	114058
	Adx	NC	▲	102532
	Adx reductase	NC	▲	104993
Ferredoxin/ Ferredoxin reduct.	NC	▲	104993	

48 h ovary

96 h ovary



Selected Pathway: Steroidogenesis-related Table
 Colored by: ZF fadrozole 48 and 96 h ovary, Default Interpretation
 Gene List: Steroidogenesis-related Table (16)

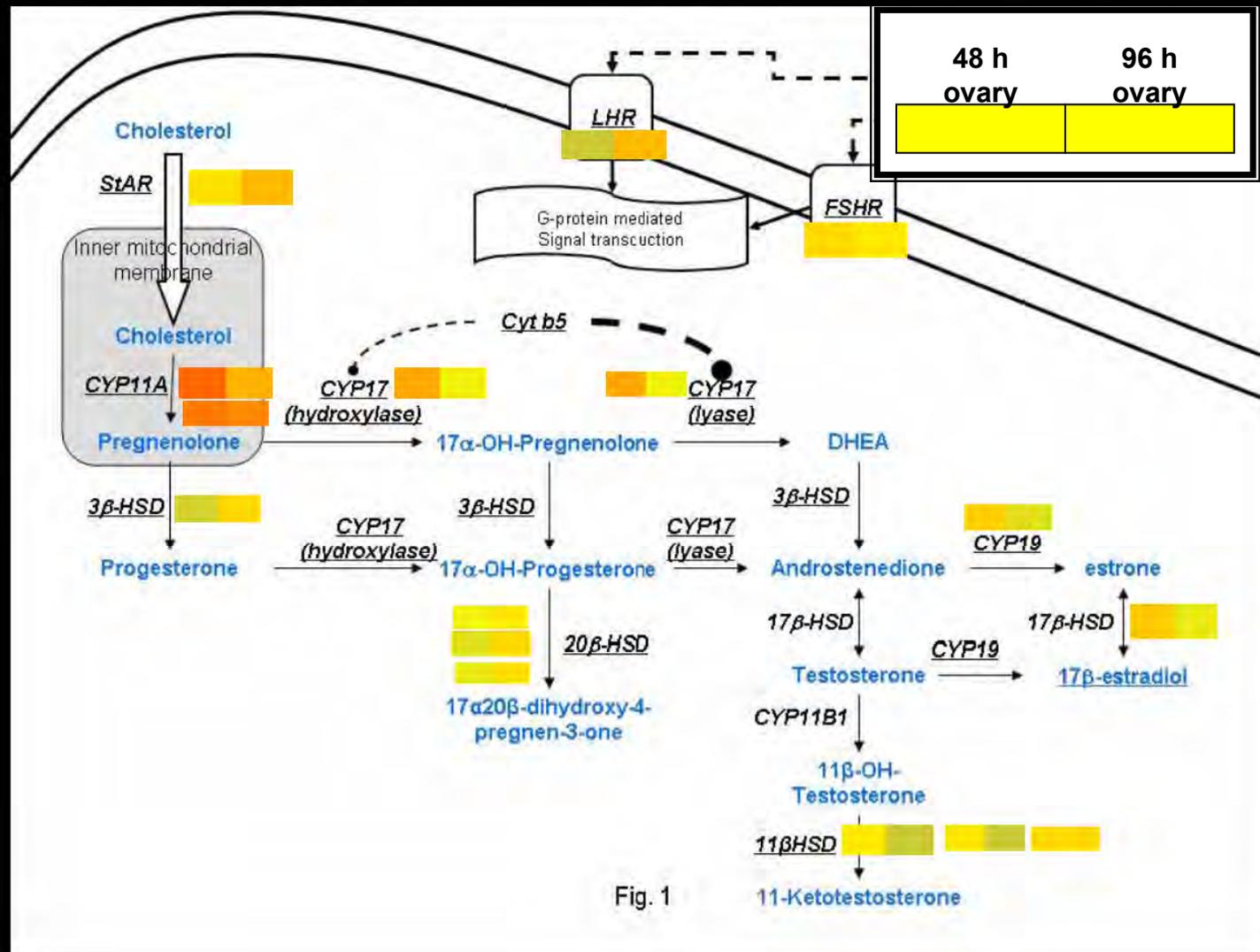


Fig. 1

Selected Pathway: steroidogenesis genes MS figure
 Colored by: ZF fadrozole 48 and 96 h ovary, Default Interpretation
 Gene List: Teleost BPG hypothesis-driven list (systems model based) (83)

Hypothesis-based approach: Model Limitations

Static

Hypothesis formulation requires time course assumptions

Genomic data represents “snap-shot” observations of the system

Concentration Dependence

Large number of endpoints – sensitivity to direct effects of stressor will vary

Secondary effects (e.g. compensation, feedback, cross-talk) – dependent on dynamic concentrations of endogenous compounds and/or structural changes in biomolecules – not readily quantified

Resolution

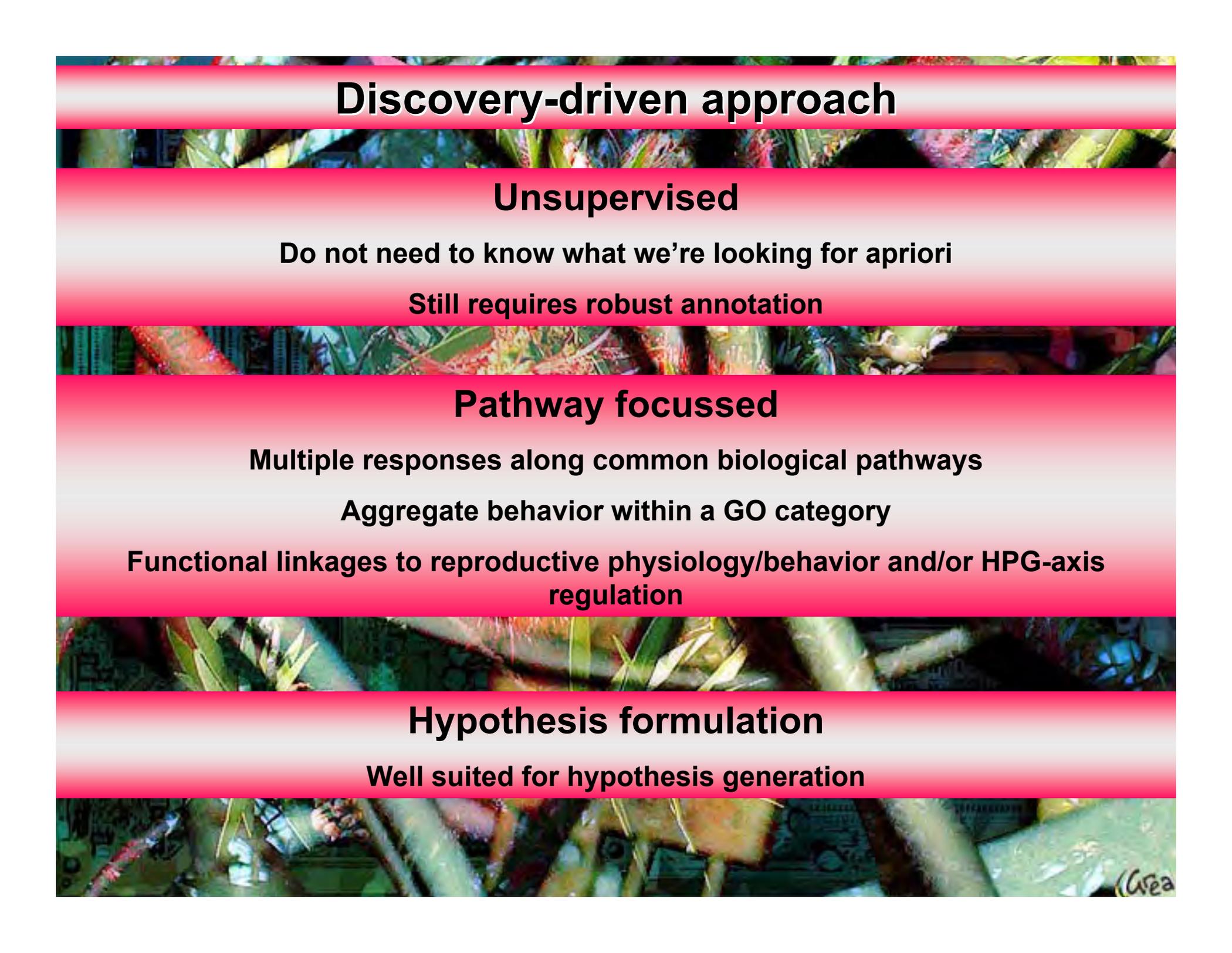
Measurements – tissue scale resolution

Function – depends on finer resolution (e.g. cell types within tissue)

Supervised

Dependent on existing information in the literature

Requires robust annotation



Discovery-driven approach

Unsupervised

Do not need to know what we're looking for a priori

Still requires robust annotation

Pathway focussed

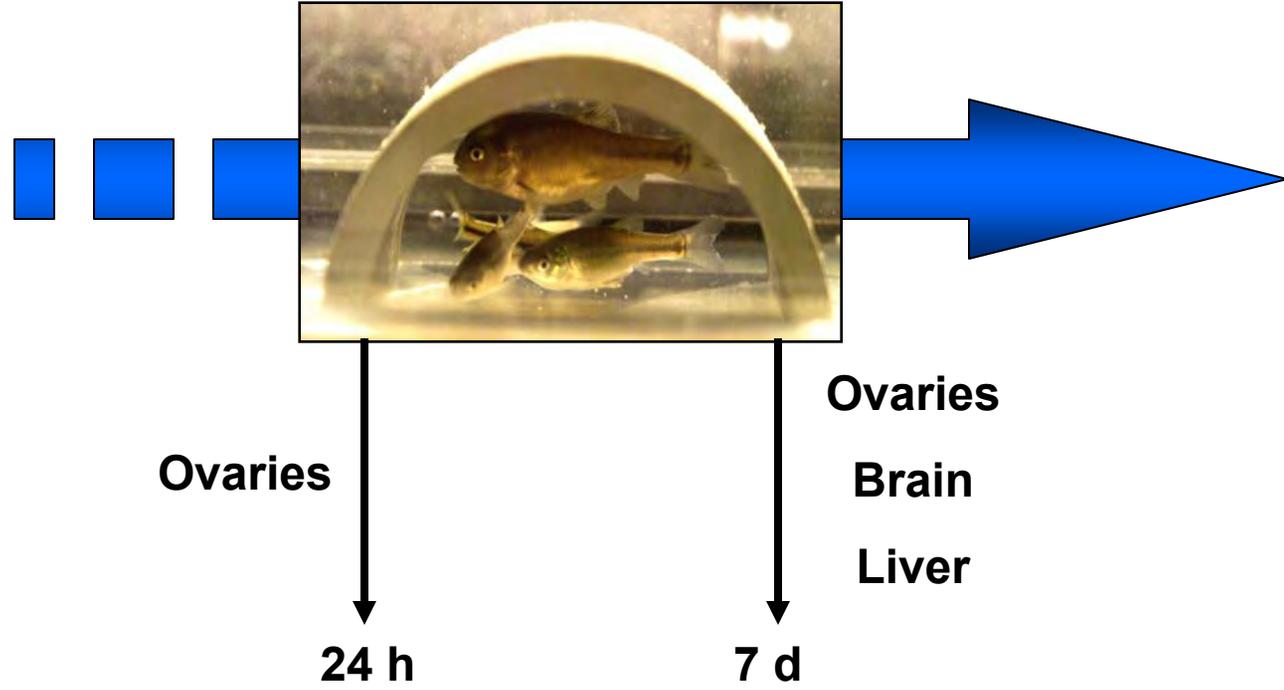
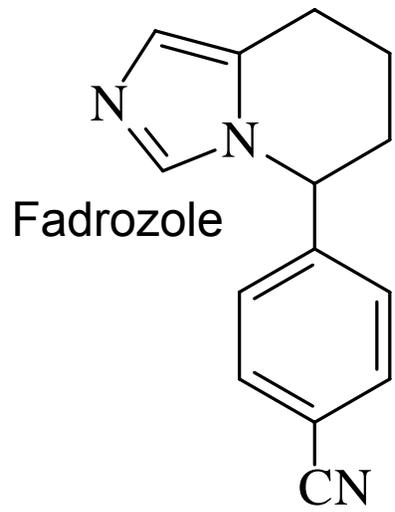
Multiple responses along common biological pathways

Aggregate behavior within a GO category

Functional linkages to reproductive physiology/behavior and/or HPG-axis regulation

Hypothesis formulation

Well suited for hypothesis generation



2,000 unique FHM genes

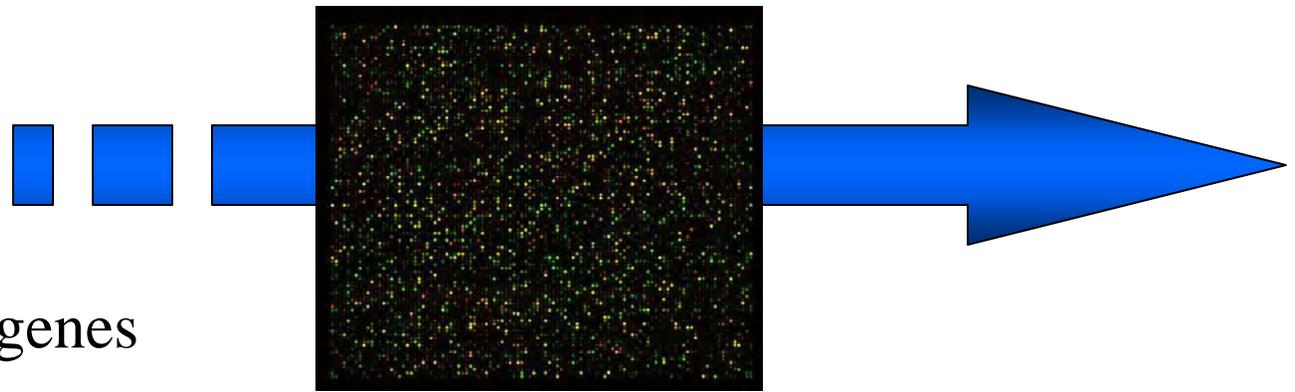
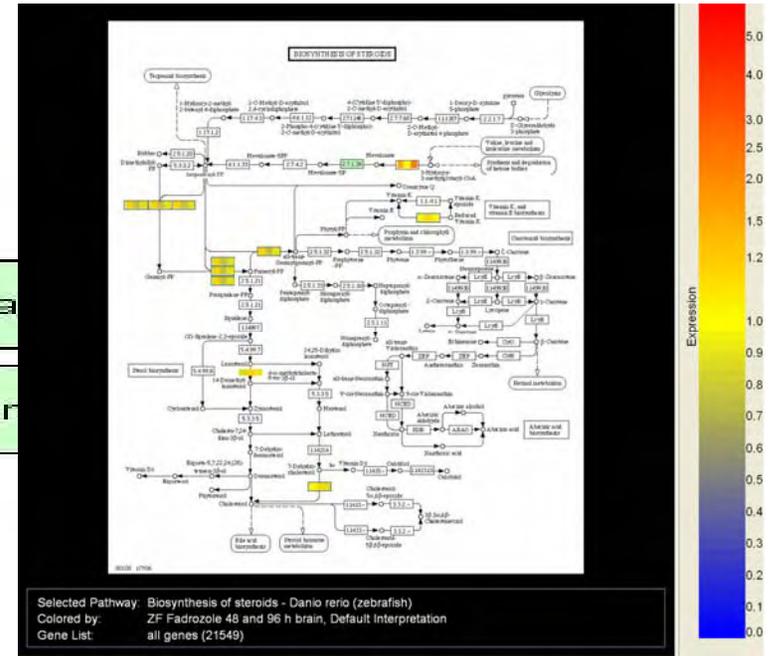
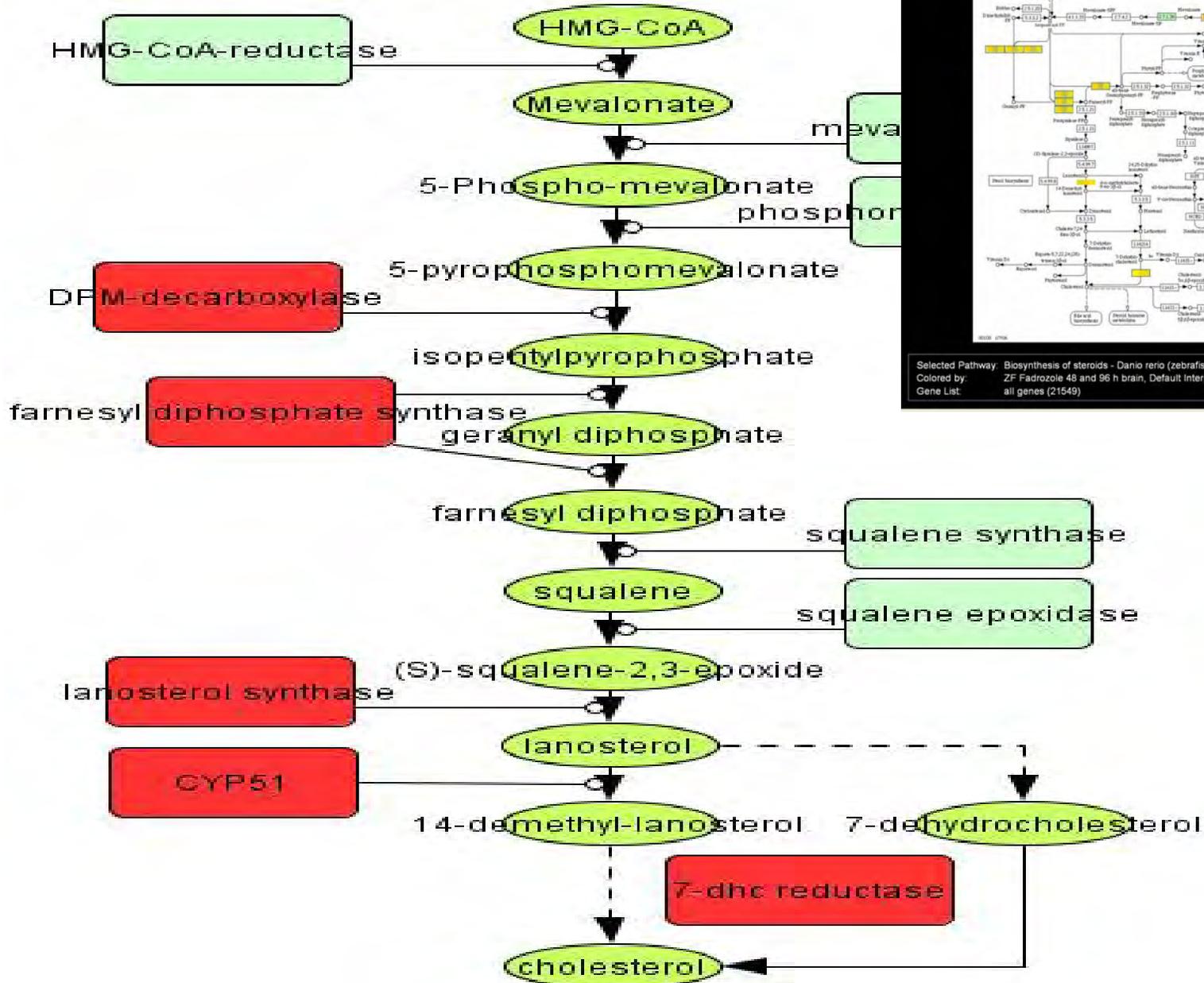
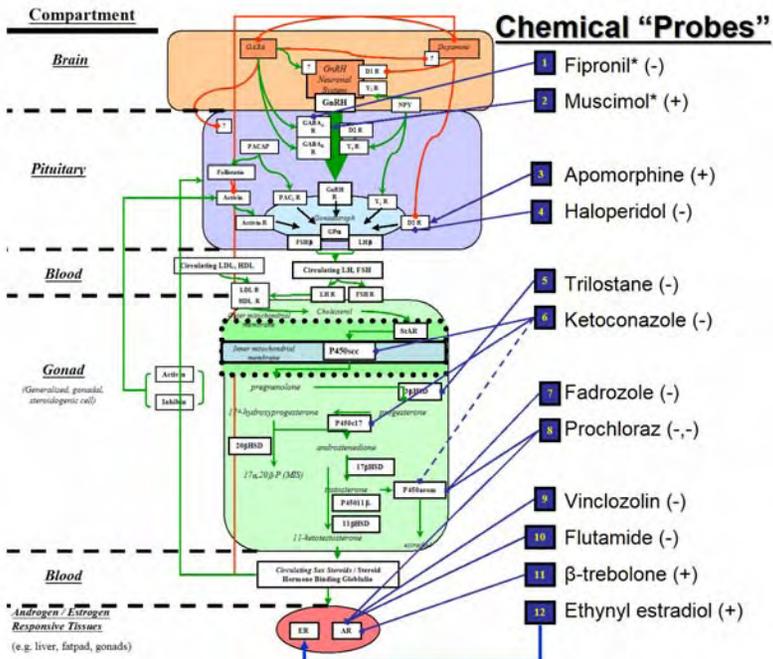


Figure 1

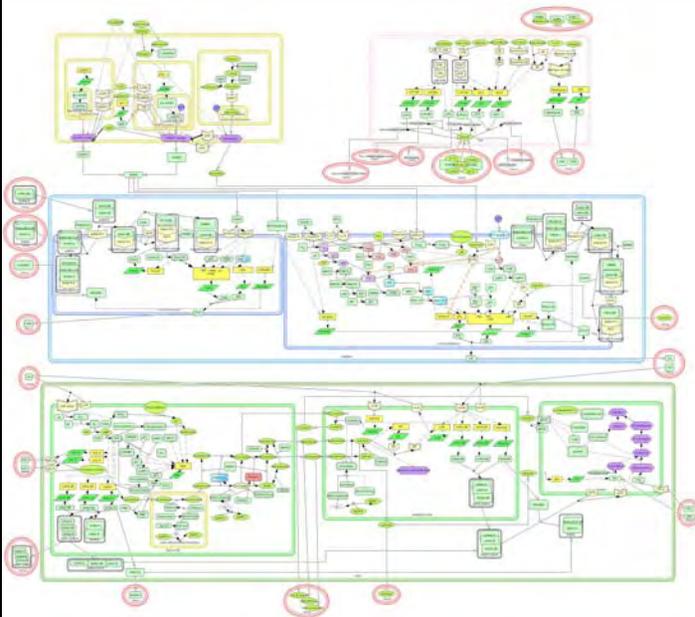




Model refinement through successive rounds of hypothesis generation and empirical testing

Model refinement through discovery of additional impacted pathways and/or functions

Refine toward greater predictive sophistication



Summary

On-going collaborative research

Linking: MOA → molecular/biochemical → ecol. relevant

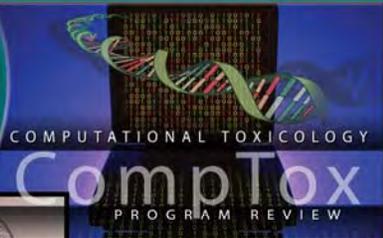
- Continuum of biological response from inhibition of steroidogenic enzyme activity to reduced fecundity
- Linkage of functionally-relevant biomarker response to predicted population impacts using a density dependent population model.
- Evidence for biological compensation to chemical-induced effects at multiple levels of biological organization.

Summary

On-going collaborative research

Linking: MOA → molecular/biochemical → ecol. relevant

- Exploring potential to use fingerprint-based biomarkers
- Refinement of our systems model and understanding through iterations of hypothesis- and discovery-based analysis
- Simultaneously generating data used to inform development of a computational mechanistic model of vertebrate steroidogenesis (Breen et al., AM) and other functions of the reproductive axis (Watanabe et al., AM)



Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models

On-going collaborative research

Linking: MOA → molecular/biochemical → ecol. relevant

- More relevant molecular markers of exposure and/or effects
- Improved understanding of biological response to stressors
- Predictive models.

Improved prospective and retrospective risk assessment



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